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PLANT AND SOIL

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AVAILABILITY OF DICALCIUM PHOSPHATE TO PLANTS, WHEN APPLIED IN VARIOUS FORMS*)

by J. HAGIN

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INTRODUCTION

Presently, the main source of phosphorus for Israel agriculture is single superphosphate. There is, however, a general trend in the fertilizer industry to produce phosphorus fertilizers by treatment of rock phosphate by nitric acid and ammonia instead of by sulfuric acid. The resultant products contain a part of the phosphorus as dicalcium phosphate. Most of the soils in Israel have a pH higher than neutral and contain appreciable amounts of calcium carbonate. It is believed, as summarized by Rogers *et al.*[9], that under such conditions phosphoric fertilizers containing dicalcium phosphate are less available than ordinary superphosphate. Starostka and Hill [12] showed that mixing dicalcium phosphate with other salts, especially ammonium sulfate, renders it more available.

The purpose of the present investigation was to examine the availability to plants of dilcalcium phosphate when applied in different forms and mixed with ammonium sulfate. Two soils were chosen for the experiment. One calcareous, with a slightly alkaline pH, and the other noncalcareous and neutral.

MATERIALS AND METHODS.

Two soils were included in the experiment: a sandy soil from Rehovot in the Coastal Plain, having a pH 7.0, noncalcareous; and a sandy loam from Gilat in the Negev region with a pH of 7.6, with 12.8% $CaCO_3$. Both soils have low amounts of organic matter, 0.5% and 0.6% respectively, and

* This research work was financially supported by Fertilizers and Chemicals Ltd., Haifa, Israel.

are non saline, having an electrical conductivity of 0.5 and 0.7 millimhos/cm at 25°C.

Analytical methods used for soils and plant materials were the same as described in an earlier paper (6).

The phosphate fertilizers used were prepared by "Fertilizers and Chemicals Ltd.," Haifa, Israel. Their description follows:

Symbol	Fertiliser	P, %		N, %
		Soluble in ammonium citrate solution.	Total	
DCP	Dicalcium phosphate in powder form	16.5	18.2	—
DCP gran.	Dicalcium phosphate + $(NH_4)_2SO_4$ granulated	12.5	13.8	6.2
Super	Superphosphate in powder form	6.9	7.5	—
Super gran.	Superphosphate + $(NH_4)_2SO_4$ granulated	6.0	6.5	3.0

The granules in the granulated fertilizers were 2–4 mm in size.

The soils were passed through a 5-mm hole sieve and transferred into greenhouse pots (7 kg per pot). The fertilizers were mixed with the upper half of the soil in each pot. The amounts of fertilizers applied were calculated on per dunam basis. It was assumed that the toplayer of one dunam (1000 m^2) contains 250 tons of soil.

The various fertilizer treatments were as given in Table I.

All treatments received 7.5 kg K per dunam as potassium chloride.

The whole experiment was performed in 4 replicates arranged as blocks in the greenhouse. The moisture content of the soils was kept approximately at "field capacity", by frequent watering with distilled water.

Clover (*Trifolium alexandrinum*) was sown in the middle of December 1956, and after emergence the plants were thinned to ten per pot. The clover was cut for the first time at the beginning of March 1957 and for the second in the middle of April. As growth preceding the second harvest was not satisfactory the second cutting was done at the soil surface, and cow peas (*Vigna sinensis*) were sown immediately after. Four plants per pot were left to grow. The cow peas were harvested in the middle of July. A few days after the cow peas were harvested, corn (*Zea mays*) was sown and thinned to four plants per pot. The corn plants were cut in the middle of October 1957, when they were in early bloom. The corn was topdressed with nitrogen fertilizers at the beginning of its growth, as the plants seemed to be nitrogen deficient. Each pot received 1 g of NH_4NO_3 in solution.

In all cases the green plants were oven dried immediately after harvest and the oven-dry weights was determined. The plants from the four replicates were combined into one sample, ground in a Willey mill, and total phosphorus in the plants was determined.

Analysis of variance of the yield data was performed according to Snedecor [11]. The calculations of yield curves and availability coefficients were done according to the publications of the Iowa Agr. Exp. Station [2], [3], [15].

Laboratory experiments without plants were also performed. In one series, 400 grams of each of the two soils were mixed with various quantities of DCP either in powder form or in granulated form mixed with $(NH_4)_2SO_4$. The mixtures of DCP and soil were put in dishes and wetted to the "field capacity" as determined for the pot experiments. After two periods of incubation the mixtures were sampled in triplicates and soluble phosphorus determined. The methods used were: water extraction [1], sodium bicarbonate [7] and sulfuric acid [14] extractions. Additionally, on the sampling dates, pH of the soils was determined by means of a glass electrode [8].

In another set of laboratory experiments the soils from Rehovot and Gilat were mixed with DCP powder, DCP granular containing $(NH_4)_2SO_4$, and with ordinary superphosphate, in such quantities that each fertilizer supplied 250 ppm P.

The mixtures were subsequently extracted by means of ion-exchange resins [10], [13]. The preparation of resins was as follows: A polystyrene cation exchanger – "Chempro C-20" – was washed with 1 *N* hydrochloride acid solution and subsequently with distilled water, in order to obtain a hydrogen saturated cation exchanger. The anion exchanger – "Duolite ES-101" – was washed with a 1 *N* sodium hydroxide solution and distilled water as to exchange all adsorbed anions for hydroxils. The resins were air-dried and 1 gram of each placed in a small nylon fabric bag, mixed and buried in the soils containing the phosphorus fertilizers. The soils were wetted to the consistency of a saturated soil paste; covered and rewetted from time to time to maintain the same consistency. At various periods of time the bags were taken out of the soils in duplicates, the soil was washed from them and the resins were placed in glass tubes and extracted with a 2 *N* solution of sodium chloride. Phosphorus was determined in the extract by the molybdenum-blue method [5].

RESULTS

Greenhouse experiments

Over-dry weights of plants after harvest are recorded in Table I. Analysis of variance of these data showed for all crops that the variance due to treatments as a whole as well as due to different soils, due to treatments within soils, and for interaction between soils and treatments within soils was significant at a 0.01 level.

Yield curves were calculated from the data obtained. It was assumed that the plants respond to increasing rates of phosphate

* The resins were supplied by the Chemical Process Company, Redwood City, California.

TABLE I

Yields of oven-dry matter of various treatments (means, g per pot.)										
Soil	Crop	P kg. per dunam	DCP powder	DCP gran*.	DCP powder + N*	Super powder	Super gran*.	Super powder + N*	$(NH_4)_2SO_4$ only	L.S.D. for two adjacent means
Gilat	Clover 1st cutting	0	3.9						4.1	
		7.5	8.3	3.8	5.1					
		15.0	10.3	3.9	6.4					
		20.0				10.2	5.9	8.5		
		30.0	11.6	4.0	7.4					1.4
	Clover 2nd cutting	0	3.9						7.1	
		7.5	8.2	6.9	9.3					
		15.0	9.4	7.3	9.7					
		20.0				9.6	10.7	8.8		
		30.0	9.7	8.7	9.9					
										2.3
	Cow peas	0	9.9						11.7	
		7.5	10.2	15.1	13.2					
		15.0	10.3	17.4	14.2					
		20.0				11.0	10.8	11.0		
		30.0	10.3	17.8	15.6					
										2.4
	Corn	0	16.2						17.7	
		7.5	18.2	16.8	16.7					
		15.0	17.6	16.0	18.7					
		20.0				17.7	16.9	17.1		
		30.0	17.6	16.5	18.3					
										1.8
Rehovot	Clover 1st cutting	0	2.1						2.3	
		7.5	7.2	4.5	4.8					
		15.0	7.5	6.3	5.4					
		20.0				6.8	8.3	7.1		
		30.0	7.6	8.2	6.2					
										1.4
	Clover 2nd cutting	0	4.5						4.4	
		7.5	5.2	5.1	4.9					
		15.0	5.4	5.3	5.2					
		20.0				5.1	5.3	5.2		
		30.0	5.4	5.4	5.3					
										2.3
	Cow peas	0	12.1						11.3	
		7.5	12.7	12.8	11.4					
		15.0	12.7	11.4	11.5					
		20.0				10.7	9.6	7.6		
		30.0	13.2	11.1	11.5					
										2.4
	Corn	0	10.6						9.9	
		7.5	14.2	16.1	16.0					
		15.0	14.4	16.1	17.2					
		20.0				15.1	14.8	13.8		
		30.0	14.4	16.3	16.4					
										1.8

* N was added as ammonium sulphate to equalize the nitrogen supply

fertilizers according to the Mitscherlich equation: $\log (A-y) = \log A - c\,(x + b)$, where y represents the yield obtained by the x amount of fertilizer; A is the maximum yield obtainable under the prevailing conditions, when x tends to infinity; c determines the slope of the curve; and b represents the amount of available nutrient in the soil in units equivalent to those of the fertilizer. Furthermore it was presumed that all forms of the DCP fertilizer would produce the same maximal yield (A) if their supply were infinite. Accordingly, concurrent Mitscherlich curves were calculated for the DCP fertilizers for each crop on each soil [2, 15]. The corresponding curves and equations are given in Figures 1*a* and *b*. Com-

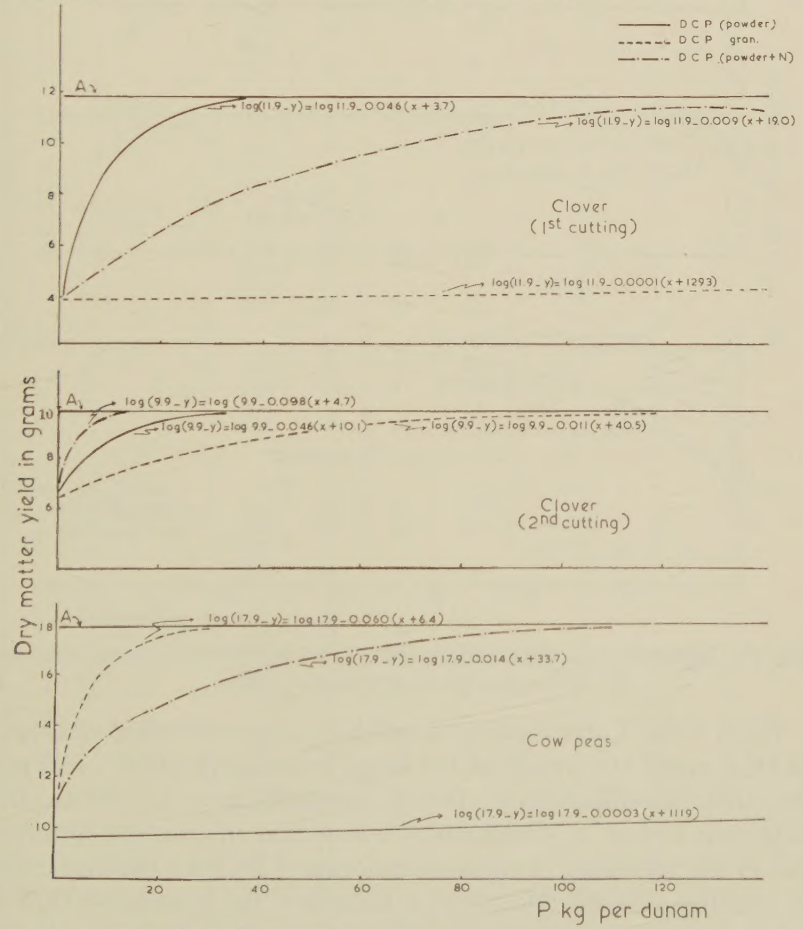

Fig. 1a. **Gilat** soil. Mitscherlich curves and equations relating amounts of P supplied, to dry matter yields.

parison of c values provides a relative evaluation of the availability of phosphates to plants in the three compounds tested. The data did not allow calculations of yield curves for cow peas on Rehovot soil and for corn on Gilat soil.

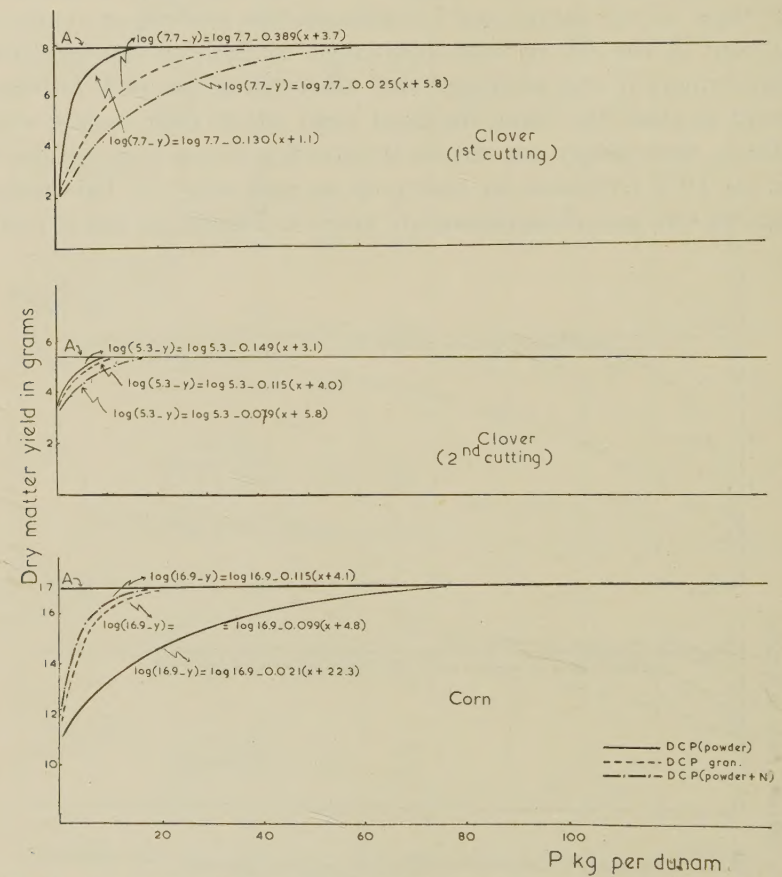

Fig. 1b. **Rehovot** soil. Mitscherlich curves and equations relating amounts of P supplied, to dry matter yields.

From Table I and Figures 1a and b it is apparent that additions of DCP raised the yields of the crops tested appreciably, and not less than superphosphate. This is especially true for clover, the first crop, tested. It is interesting to note that there are no differences in the efficiency of the various forms of DCP for the first crops in the Rehovot soil. However, in Gilat soil, the granulated DCP is inefficient in the first crop; for the second and third its relative

efficiency rises steeply, and its residual effect for cow peas is even greater than that of superphosphate.

Phosphate in the dry matter of plants was determined and yield of phosphorus per pot (mean per treatment) was calculated from the data obtained and from yield data recorded in Table I. The results are given in Table II.

TABLE II

Phosphorus yield from various treatments (means, mg per pot)

Soil	Crop	P kg. per dunam	DCP powder	DCP gran.	DCP powder + N	Super powder	Super gran.	Super powder + N	$(NH_4)_2SO_4$ only
Gilat	Clover 1st cutting	0	11						10
		7.5	19	11	14				
		15.0	30	11	13				
		20.0				32	16	21	
		30.0	25	9	21				
	Clover 2 nd cutting	0	9						8
		7.5	24	14	18				
		15.0	24	16	17				
		20.0				28	15	22	
		30.0	35	17	26				
	Cow peas	0	20						21
		7.5	22	16	26				
		15.0	27	27	27				
		20.0				25	19	15	
		30.0	30	27	36				
	Corn	0	22						19
		7.5	35	31	33				
		15.0	32	27	35				
		20.0				42	30	39	
		30.0	33	25	37				
Rehovot	Clover 1st cutting	0	4						5
		7.5	38	15	29				
		15.0	25	25	32				
		20.0				52	71	48	
		30.0	54	38	39				
	Clover 2nd cutting	0	2						4
		7.5	25	24	25				
		15.0	33	27	32				
		20.0				38	34	36	
		30.0	34	28	33				
	Cow peas	0	13						9
		7.5	34	34	55				
		15.0	72	48	67				
		20.0				76	77	65	
		30.0	91	62	94				
	Corn	0	21						19
		7.5	50	26	45				
		15.0	56	51	71				
		20.0				55	74	80	
		30.0	79	72	94				

The yield of phosphorus from the crops on the Rehovot soil shows that in almost all cases the crops could utilize the phosphates from DCP no less efficiently than from the superphosphate; furthermore, no appreciable differences could be found between the three forms tested. The same is true for the soil from Gilat, except that the granulated form was less effective for the first two crops than the other forms.

Laboratory experiments.

Mixtures of each of the two soils with DCP powder and DCP granular were prepared and extracted. The extraction results are given in Table III. The replicates of extractions results from soils containing the DCP in powder form were very uniform and only the means are presented. In the case of DCP in granular form, variation in results was encountered. In order to express the variability, standard error of the mean was calculated and given in the table in parentheses. Table III shows that even after a period of two months much of the phosphate added as DCP is extractable. It is very striking that in all cases much more phosphorus is extracted when applied in powder form than in the granulated one. It seems that granulation rendered the compound less soluble.

Parallel to determinations of soluble phosphorus after incubation, pH was measured in the soil-DCP mixtures (Table IV). In the Rehovot soil mixed with granular DCP, which contains $(NH_4)_2SO_4$, a sharp change in pH occurred. The low pH after incubation is probably due to the oxidation of ammonia. The soil from Gilat contains large amounts of $CaCO_3$ which presumably neutralized the nitric acid produced.

The results of extraction of phosphorus by ion exchange resins are given in Figure 2. The amount of phosphate adsorbed on the resin is dependent on the concentration of phosphate in the soil solution and on the rate of diffusion of this solution into the resin bed. From the results in Figure 2 it seems that the rise of the curve in the Rehovot soil during the first two weeks was mainly due to the diffusion of phosphate into the resins. After that period, an equilibrium between the outer and inner solution was presumably attained, and no further changes in the phosphate concentration could be noted. However, the curve for superphosphate shows an initial rise, similar to the DCP curves, although instead of

TABLE III

Soil	P added, ppm	Soluble phosphorus (ppm) in various extractants after two periods of incubation											
		H_2O extract				$NaHCO_2$ extract				H_2SO_4 extract			
		After 3 days		After 65 days		After 3 days		After 65 days		After 3 days		After 65 days	
		DCP powder	DCP gran.	DCP powder	DCP gran.	DCP powder	DCP gran.	DCP powder	DCP gran.	DCP powder	DCP gran.	DCP powder	DCP gran.
Gilat	0	1				8				32			
	120	39	4(±0.4)	16	5(±1)	68	37(±16)	103	19(± 3)	104	52(± 2)	97	45(±4)
	240	67	9(±3)	43	6(±1.5)	137	34(± 3)	162	20(±2)	194	55(± 4)	149	60(±1)
	480	161	9(±0.8)	107	6(±0.2)	178	76(±12)	351	52(±14)	238	64(± 3)	286	70(±5)
Rehovot	0	3				8				31			
	120	52	11(± 3)	60	13(± 3)	84	16(± 1)	124	19(± 1)	135	38(± 5)	164	43(±3)
	240	95	56(±21)	122	44(±20)	154	60(±12)	218	49(±21)	279	98(±34)	295	52(±8)
	480	187	58(± 8)	208	59(±28)	293	96(±15)	343	103(±28)	473	59(±16)	497	63(±6)

TABLE IV

pH in mixtures of soils and DCP after two periods of incubation.						
Soil	P added in ppm	Initial	After 3 days		After 65 days	
			DCP powder	DCP gran.	DCP powder	DCP gran.
Gilat	0	7.6				
	120		7.5	7.5	7.3	7.3
	240		7.5	7.5	7.3	7.3
	480		7.5	7.5	7.2	7.2
Rehovot	0	7.0				
	120		7.0	6.9	6.7	5.9
	240		7.0	6.7	6.7	4.9
	480		7.0	6.6	6.6	4.9

leveling off in the latter period it drops, which suggests a mechanism of phosphate fixation by the soil. In the Gilat soil the superphosphate curve is partly similar to that in the soil from Rehovot. There is a sharp rise and fall in the phosphate concentration, but in the latter part, the curve attains an equilibrium stage. For the DCP compounds we found a similar initial concentration of phosphate in both soils. However, in the Gilat soil the phosphate concentration drops to a low level in the latter part. It should be noted that the final concentrations of phosphate are much higher in the Rehovot soil than those in the soil from Gilat.

DISCUSSION AND CONCLUSIONS.

The most striking result of this experiment is that dicalcium phosphate showed approximately the same efficiency as phosphate fertilizer, as did the superphosphate. This does not seem surprising for the neutral and noncalcareous soil from Rehovot. However, it is not in accordance with the general experience [4, 9] for the slightly basic, calcareous soil from Gilat. Extractions of soil-fertilizer mixtures showed also, that a great part of the phosphate added as dicalcium phosphate is extractable after a period of time, *e.g.* it is not strongly fixed by the soil particles. The results of the experiment with resins suggest that for a short period of time there is more phosphate in the soil solution in cases where it was supplied as superphosphate than where it was given as dicalcium phosphate. However, after that time a large part of the soluble phosphate is

fixed, and the phosphate concentrations are approximately equalized in the soil solutions from both sources. This could also serve

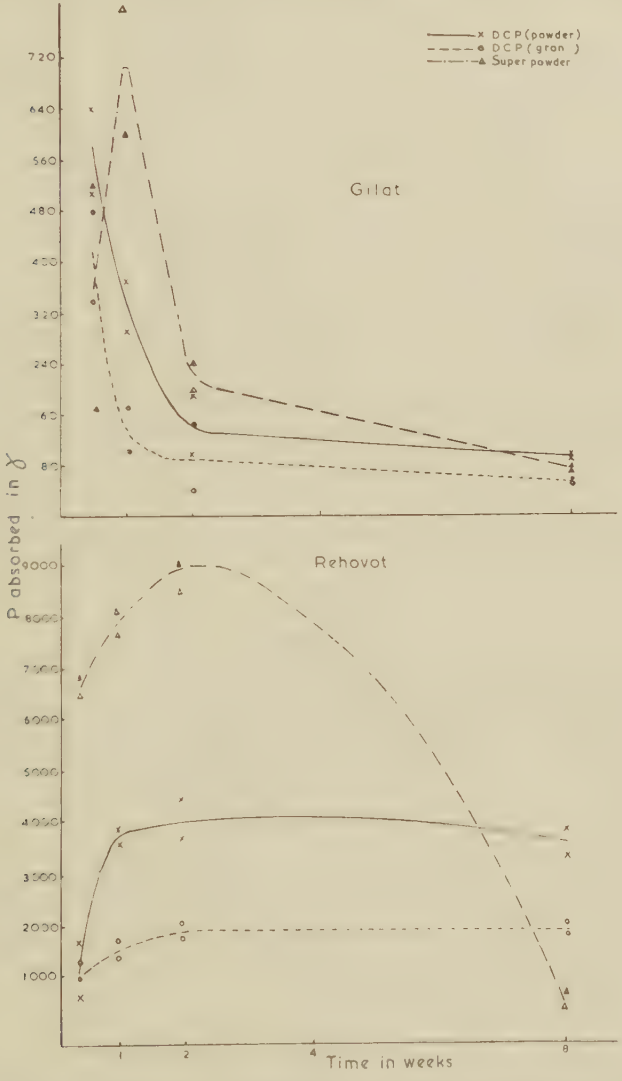

Fig. 2. Adsorption of phosphates by anion exchange resin from soil-fertilizer mixtures, as a function of time.

as an explanation why the dicalcium phosphate was as efficient as superphosphate in supplying phosphate to plants.

Addition of ammonium sulfate to the dicalcium phosphate did not enhance the phosphate availability to plants. On the contrary, for the first crops on the Gilat soil, phosphate availability was lowered. However, it seems that the factor that influenced its solubility was probably the granulation process and not the addition of ammonium sulfate. Nitrogen by itself had very slight influence on yields. This is demonstrated by the yields obtained from various control treatments containing ammonium sulfate. Laboratory experiments also showed that the dicalcium phosphate was less soluble when applied in granulated form. This finding is in accordance with conclusions of other authors.[4]

The practical conclusion to be drawn from these experiments is that the emphasis on supply of water-soluble phosphorus fertilizers to calcareous soils should be revised. The scope of the above experiment is relatively very small and no final positive conclusions on suitability of citrate-soluble phosphate for fertilizing calcareous soils should be drawn. However, it shows that there is a possibility that the citrate-soluble compounds might be used as phosphate fertilizers and that further research on a larger scale is desirable.

SUMMARY

The availability of dicalcium phosphate in powder and granulated form mixed with ammonium sulfate, was tested in a greenhouse experiment. Two soils were used in the experiment: one from Rehovot, neutral and non calcareous, and the other from Gilat, slightly alkaline and calcareous. Clover (two cuttings) was the first crop and cow peas and corn succeeded. The yield results were fitted to Mitscherlich curves. In general, the results of greenhouse and laboratory experiments showed, that in both soils the dicalcium phosphate had approximately the same efficiency as phosphate fertilizer, as did superphosphate. It appears that additions of ammonium sulfate had no appreciable effect on phosphate availability. However, the granulation process lowered its solubility. It may be concluded that there is a possibility that citrate-soluble phosphate could be efficient on calcareous soils no less than the water-soluble form.

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DIE PRÜFUNG VON BODENFUNGICIDEN

I. PILZ – SUBSTRAT – FUNGICID – KOMBINATIONEN

von KLAUS H. DOMSCH

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EINLEITUNG

Zur Bekämpfung von Bodenpilzen in wertvollen Kulturen werden in zunehmendem Maße organische Fungicide herangezogen, die sich als Spritzmittel zum Schutze oberirdischer Organe bewährt haben, oder die eigens zu diesem Zweck hergestellt wurden. Damit ist eine Entwicklung eingeleitet, deren Ursprung in der Ausdehnung des Beizgedankens und in den Bemühungen um eine chemische Bodenentseuchung liegt. Vom biologischen und pflanzenhygienischen Standpunkt aus kann dieser Prozeß nur in sehr engen Grenzen gutgeheißen werden. Deshalb erfordert das Gebiet der Bodenfungicide gründliche Bearbeitung, um aus sicherer Kenntnis der Grundlagen Einfluß auf Richtung und Intensität der angedeuteten Entwicklung nehmen zu können.

I. MATERIAL UND METHODEN

a. *Pilze.*

In die Untersuchungen wurden folgende Pilze einbezogen: *Fusarium culmorum, Pythium* sp., *Rhizoctonia solani* und darüber hinaus für einige vergleichende Versuche: *Alternaria brassicae, Aspergillus niger, Botrytis cinerea, Thielaviopsis basicola.*

Kultivierung auf komplexem Nähragar (1,5% Agar; 2% Malzextrakt; 0,5% Pepton), tägliches Überimpfen. Von langsam wachsenden, reichlich sporulierenden Pilzen wurden Sporen zusammen mit dem Nähragar in eine Schale ausgegossen und nach gleichmäßigem Bewuchs der Platte als Inoculum verwendet.

b. *Fungicide.*

N-Trichlormethylthiotetrahydro-phthalimid (Captan), aktiver Wirkstoff 50%, Anwendung in wässriger Suspension.

Tetramethylthiuramdisulfid (TMTD), aktiver Wirkstoff 80%, Anwendung in wässriger Suspension.

8-Oxychinolinsulfat (Kaliumsulfat), aktiver Wirkstoff 51%, Anwendung als wässrige Lösung, Komplexbildner.

c. *Testverfahren.*

Die allgemein üblichen Verfahren zur Prüfung von Blattfungiciden (Sporentests, vgl. Horsfall [11]) sind für die Prüfung von Bodenfungiciden wenig oder sogar ungeeignet. Die vielgestaltige Problematik hat zu zahlreichen Methoden (Tab. I) geführt, bei denen in jedem Einzelfalle auf Vereinfachungen nicht verzichtet werden kann, aus denen sich aber die folgenden wichtigen Grundprinzipien ableiten lassen:

1. Reaktionen des Mycels müssen als Bewertungskriterien im Vordergrund stehen.

2. Bodenpilze wachsen substratgebunden. Labortests, bei denen mit weitgehend freien Mycelien gearbeitet wird, sind zur Prüfung von Bodenfungiciden nicht brauchbar.

3. Die besonderen, im Boden ständig vorliegenden abiotischen Bedingungen (Filterung von Suspensionen, Adsorption, Bodenreaktion u.a.) müssen im Test berücksichtigt werden.

Im Verlauf der eigenen Untersuchungen wurden die folgenden Verfahren angewandt.

α) Flüssigkeitstest: Nährlösung (2% Malzextrakt; 0,5% Pepton) mit abgestuft konzentrierten Fungicidansätzen vermischt; Beimpfung mit Mycelscheiben; Anfangs-pH 6,2; Bebrütung bei 20 C; Auswertung nach 10 Tagen. Impfstücke ohne sichtbares Wachstum dem Kolben entnommen, gewaschen, Wachstum auf unbegiftetem Agar beobachtet. Je Konzentration 3-fache Wiederholung.

β) Agar-Test: In großen Reagenzgläsern Malzextrakt-Pepton-Nährboden mit jeweils abgestuften Fungicidansätzen gemischt, in Schalen ausgegossen, beimpft und bei 20 C bebrütet. Messung der Koloniedurchmesser. Je Konzentration 5-fache Wiederholung.

γ) Schalen-Test: *Sandschalen* (vgl. Domsch [5]): In je eine Petrischale 40 g (= 25 ccm) gewaschener, lufttrockener Seesand eingewogen und heiß sterilisiert. Herstellen von Nährlösung-Fungicid-gemischen wie unter (α), Zugabe von 8 ccm dieses Gemisches pro Sandschale und gründliche Verteilung. Relat. Wasserkapazität etwa 80%, Anfangs-pH ca. 6,2; Bebrüten und Auswerten der Schalen wie unter (β). *Torf/Sand-Schalen:* (Domsch[5]): Folgende Volumina (bzw. deren Vielfache) untereinander vermischt: 90 ccm Torf (lufttrocken, fein gesiebt), 210 ccm Seesand, 30 ccm einer Lösung mit 6% Malzextrakt und 1,5% Pepton, 10 ccm *N*/1 NaOH, 20 ccm Wasser. Von dem

TABELLE I

Zusammenstellung einiger Prinzipien zur Fungicidprüfung mit Mycelien.	
Autoren	Testprinzip
1. Davey and Leach [3]	Sklerotien von *Sclerotium rolfsii* in Beuteln in fungicide Flüssigkeiten eingehängt oder vergraben, Boden mit Fungicid behandelt, anschließend Beobachtung auf Agar.
2. Evans [6]	Verwendung von „recolonization tubes" (Evans 1954), Vergleich von Erd- und Agarsubstrat.
3. Ezekiel [7]	Vorarbeiten für Methode 12; u.a. Einbringung des Pilzes in den Boden nach Applikation des Mittels.
4. Forsberg [9]	Pilzbewachsener Baumwollfaden wird mit Fungicid bestäubt oder benetzt, anschließend Beobachtung auf Agar.
5. Jacks and Smith [13]	Pilzbeimpfte Agarplatten werden in hohen Gläsern der Gasphase von Fungiciden ausgesetzt.
6. Mason and Powell [18]	Pilzbewachsene Weizenkörner in fungicide Flüssigkeit eingehängt, Wachstumsmessungen, Vergleich mit Sporenkeimungstest.
7. Meredith [22]	Trockener Boden in Röhrchen, Fungicid aufgetropft, Pilz aufgeimpft, Wachstum beobachtet.
8. Roberts and Bollen [26]	Auszählen von Pilzkolonien in Ausstrichen einer Bodenaufschwemmung nach Fungicidbehandlung.
9. Strecker [29]	U.a. Mycelhomogenisate im Rollkulturverfahren geprüft.
10. Vaartaja [30]	Pilzbewachsene Agarplatte mit Fungicidagar überschichtet, darauf Samen ausgelegt. Gleichzeitige Bewertung fungicider und phytotoxischer Merkmale.
11. Wilhelm and Ferguson [31]	Pilzbewachsene Pflanzenteile in erdgefüllte Kanister, nach Fungicideinwirkung abgesiebt, dann Beobachtung auf Agar.
12. Zentmyer [32]	Vg. „Z-Test" (Seite 116).

Gemisch pro Schale 40 g abgefüllt und sterilisiert, Anfangs-pH 5,6; relat. Wasserkapazität etwa 70%. Nach Abkühlen 8 ccm Fungicidansatz zugegeben, Weiterbehandlung wie im Schalentest mit Sand, je Konzentration 5-fache Wiederholung.

δ) Z-Test: Die von Zentmyer[32] veröffentlichte Methode wurde in folgender Weise abgewandelt: In sterile Glaszylinder (h = 70 mm, Ø = 25 mm) 15 ccm steriles Substrat (z.B. Sand, Torf/Sand-Gemisch, Komposterde) eingefüllt,

Mycelscheibe (Ø 10 mm) des Testpilzes aufgelegt, gleiche Menge Substrat nachgefüllt und pro Röhrchen 8 ccm Fungicidansatz aufgetropft, relat. Wasserkapazität dann etwa 90%. Bebrütung für 24 h bei 20°C., dann Testpilz entnommen und Wachstum auf unbegiftetem Agar beobachtet, Bestimmung der Aufwandmenge, bei der kein Auswachsen aus dem Impfstück mehr erfolgt. Je Konzentration 3 Wiederholungen.

ε) P-Test: Unter Einbezug eines Testverfahrens von Park [23] Erweiterung des Z-Tests für quantitative Bestimmungen, Messung der Kolonie-Ø nach Wachstum auf dem unbegifteten Agar. Das Prinzip liegt hier in der Teilvergiftung des Inoculums durch sub-toxische Fungicidkonzentrationen, die sich in einer anfänglichen Wachstumsverzögerung äußert. Die Längeneinheit, um die das teilvergiftete Impfstück hinter der Kontrolle zurückbleibt, ist im Laufe des Versuches konstant; eine Signifikanz der prozentualen Hemmung wird nur bei schnellwachsenden Pilzen erreicht. Je Konzentration 5-fache Wiederholung.

ζ) Sporentest: Herstellen der Agarplatten wie unter (β), Ausstreichen einer Sporensuspension auf der Agarschicht, Auszählen der Keimprozente nach 16–20 Stunden.,

d. *Auswertung und statistische Bearbeitung.*

Die Auswertungsmethode richtet sich nach der Art der gewünschten Information. Fungicide Wirkstoffe lassen sich im wesentlichen durch die Höhe der fungistatischen oder -toxischen Grenzkonzentration kennzeichnen oder durch ED-Werte (= effektive Dosis, vgl. Horsfall [11]), einschließlich der Neigung ("slope") von Regressionsgeraden, die sich aus Dosis-Wirkung-Relationen ergeben *. Eine Grenzkonzentration wird als fungistatisch angesehen, wenn der noch lebensfähige Pilz auf begiftetem Substrat kein meßbares oder sehr schwaches Wachstum zeigt; sie wird als fungitoxisch bezeichnet, wenn der Pilz nach der Fungicideinwirkung auch auf unbegiftetem Substrat nicht mehr wächst.

Sehr ergiebig sind Auswertungsverfahren, bei denen die gesamte Skala der pilzlichen Wachstumsreaktionen für eine abgestufte Konzentrationsreihe messend verfolgt wird (Agar-, Schalen-, P-Test). Die Wachstumshemmung der einzelnen Versuchsglieder wird dann in Prozent der Kontrolle festgelegt, um auf diese Weise mit einer Größe, die den Hemm ("ng")-Prozenten bei Sporentesten entspricht, in die Probitanalyse (Finney [8]) eintreten zu können. Bei Förderungseffekten (Schultz-Arndt'sche Regel **) wurden alle Werte, die über der Kontrolle lagen, aus der Berechnung fortgelassen.

Die Bestimmung von ED50- bzw. ED90-Werten erfolgte rechnerisch †,

* Über die Bedeutung von Dosis-Effekt-Kurven für die Beurteilung von Fungiciden vgl. Dimond *et al.*[4], Martin *et al.* [17], McCallan *et al.* [21].

** Vgl. hierzu auch Angaben von Gibson [10] und von Jaenichen und Heimann [14] für Hg-Präparate bzw. 8-Oxychinolinsulfat.

† Für freundliche Hinweise sei den Herren Dr. Finney (Aberdeen) und Dr. Kundert (Wädenswil) auch an dieser Stelle vielmals gedankt.

da für den exakten Vergleich verschiedener Prüfverfahren Angaben über die mittlere Abweichung der ED-Werte nützlich sind. Die statistische Analyse richtete sich nach folgendem Arbeitsgang:

1. Berechnung des Regressionskoeffizienten b nach

$$b = \frac{Sxy - (\bar{x}\, Sy)}{Sx^2 - (\bar{x}\, Sx)}.$$

In der Gleichung bedeuten Sx = Summe aller Logarithmen der angewandten Konzentrationen; $\bar{x}$ = das arithmetische Mittel aus Sx; Sy = Summe aller Probit-Werte; $\bar{y}$ = das arithmetische Mittel aus Sy.

2. Aus der Regressionsgleichung

$$y = \bar{y} + b\,(x - \bar{x})$$

ergibt sich für $y = 5$

$$x = \frac{5 - \bar{y}}{b} + \bar{x}$$

Der Nummerus von x ist der gesuchte ED50–Wert.

3. Die mittlere Abweichung von ED50 wurde berechnet nach

$$S^2_{ED50} = \frac{S(y - \bar{y})^2 - [b^2 \,.\, S(x - \bar{x})^2]}{b^2(M - 2)} \left[\frac{1}{M} + \frac{(ED50 - \bar{x})^2}{S(x - \bar{x})^2}\right].$$

In der Gleichung bedeuten: M = Anzahl der Wertepaare xy.

4. Die Signifikanz der Differenz zwischen zwei ED50-Werten wurde mit Hilfe des t-Tests überprüft. Den in der Toxikologie üblichen Forderungen folgend wurde bei einem $P \leq 0{,}05$ eine Differenz zwischen zwei ED50-Werten als signifikant angesehen.

5. Die Signifikanz der Differenz zwischen zwei Regressionskoeffizienten wurde nach Linder [16] ermittelt.

IIA. VORVERSUCHE

a. *Typ der Regressionsgeraden.*

Da bei Fungicidtests Abweichungen vom Normaltyp der Regressionsgeraden nicht selten sind, erwies sich eine graphische Vorprüfung der erhaltenen Meßwerte als notwendig. Charakteristisch „gebrochene" Regressionsgeraden zeigt Abb. 1 *.

„Invertierte" (TMTD)-Kurven (vgl. Horsfall [11]) haben auf komplexen Substraten im Myceltest so stark abgeflachte Maxima und Minima, daß das Inversions-Phänomen nur selten deutlich in Erscheinung tritt. Im Zusammenhang mit der Auswertung von Fungicidtests muß hier auf folgende Gesichtspunkte besonders hingewiesen werden:

* Weitere Mitteilungen über diesen Typ erfolgen a.a.O.

1. Extrapolationen zur Ermittlung von ED-Werten sind nur zulässig, wenn die Geradlinigkeit der Dosis-Wirkungs-Geraden für die jeweils vorliegende Wirkstoff-Testpilz-Kombination erwiesen ist.

2. Die Skala der Test-Konzentrationen soll Aussagen über die Hemmwirkung im Bereich von ED10 bis ED 90 zulassen. Je enger der gewählte Konzentrationsbereich, desto unsicherer die Information.

3. Die Auswertungsmethode muß so genau sein, daß begründete Abweichungen vom Normaltypus der Regressionsgeraden nicht durch die Streuung der Meßwerte überdeckt werden.

b. *Mycel- und Sporenempfindlichkeit.*

In dem in Tabelle II wiedergegebenen Versuch sind ED50-Werte von Sporen und Mycelen gleicher Pilze gegenübergestellt *. Die Unterschiede in der Empfindlichkeit sind sehr groß, sie weisen hohe Signifikanz auf und die Mycelempfindlichkeit ist offenbar kein konstantes Vielfaches der Sporenempfindlichkeit. Wir dürfen also zu Recht folgern, daß Prüfverfahren, die auf Sporenkeimung abgestellt sind, für die Bearbeitung der vorliegenden Fragen ungeeignet sind **.

TABELLE II

Vergleich von Sporen- und Mycelempfindlichkeit gleicher Pilze gegenüber Captan auf begiftetem Substrat. ED50-Werte in ppm (aktiver Wirkstoff). $P < 0{,}0027$ bei allen Pilzen.

Pilz	Sporen	Mycel
Alternaria brassicae	16,2 ± 5,2[+]	[illegible]
Aspergillus niger	4,1 ± 12,9	77,8 ± 2,3
Botrytis cinerea	6,2 ± [illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]

[+] Mittlere Abweichung in Prozent des Mittelwertes

c. *Mycelalter.*

Die für *Pythium* sp., *Rhizoctonia solani* und *Fusarium culmorum* angesetzten Versuche mit verschieden alten Inocula (1, 2, 4, 8

* Weitere Teilbeiträge zu dieser Frage liefern die Arbeiten von Rich [24], Sijpestein and van der Kerk [27], Strecker [28], sowie McCallan and Miller [19].

** Hinzuweisen ist auch auf die Untersuchungen von Horsfall and Rich[12] und von Rich [25] über die Wirkung spezifischer Sporen- und Mycelinhibitoren, sowie auf die verschiedenen Glukoseabbauwege in Sporen und Mycelien von *Fusarium solani* (Cochrane [2]).

und 15 Tage) ergaben, daß die Empfindlichkeit der Pilze gegenüber einer geprüften mittleren Fungicidkonzentration bis zu einem Alter von 8 Tagen keine signifikante Altersabhängigkeit zeigt, sofern der fungicide Effekt in Prozent des jeweiligen Kontrollwachstums ausgedrückt wird. 15 Tage alte Mycelien zeigen eine leicht erhöhte Empfindlichkeit. Dieser Befund entspricht einer Mitteilung von Zentmyer und Klotz [33] für *Trichoderma lignorum* und *Botryosphaeria ribis*.

d. *Art des Inoculums.*

Die Giftempfindlichkeit ist häufig abhängig von der allgemeinen Disposition des Organismus. Es wurde daher im Z-Test geprüft, ob einerseits bei Veränderung des Nährstoffgehalts im Impfstück (Agarscheibe mit 0,1—2% Malzextrakt) und andererseits bei stofflich verschiedenem Impfmaterial (Gazestückchen, Schnurstücke, Blattstiele, Blattscheiben, Strohhalm mit Pilzbewuchs in vergleichbarem Alter) eine Verschiebung der fungitoxischen Grenzkonzentration zu beobachten ist. Aus den Versuchsergebnissen dürfen folgende Schlüsse gezogen werden:

1. Der Nährstoffgehalt des Impfstückes bleibt innerhalb der Grenzen des Versuches ohne Einfluß auf die Fungicidempfindlichkeit.

2. Da der Pilz auf „perforierten" Substraten (Gaze) erst bei höheren Fungicidaufwandmengen abgetötet wird als auf „kompakten" Agarscheiben, dürfte der mechanische Widerstand einer Agarscheibe für die Akkumulation eindringender Suspensionsteilchen von geringer Bedeutung sein.

3. Je höher der Trockensubstanzgehalt des Substrates ist, das vom Pilz direkt besiedelt wird, desto stärker ist wahrscheinlich das Eindringen schwerlöslicher Wirkstoffe behindert und desto schwerer ist also die Abtötung des Mycels im Substrat *. Dieser Befund ist bei der Übertragung der Ergebnisse von Laborversuchen auf Gewächshaus- oder Freilandverhältnisse zu beachten.

e. *Sterilisation.*

Für das optimale Wachstum von Mycelien, das sich meist über mehrere Tage erstreckt, müssen im Laborversuch Bedingungen

* Stark [28] vergleicht pilzbewachsene Torfmull- und Agarstücke und kommt zu prinzipiell gleichen Ergebnissen.

geschaffen werden, die im Gegensatz zum Sporentest eine Reihe unvermeidlicher Belastungen in der Versuchsanlage mit sich bringen. So ist neben einer ausreichenden Versorgung mit C- und N-Quellen eine Sterilisation der Substrate oft unerläßlich. Eine Erhitzung der Wirkstoffe ist zu vermeiden, da die Wirkung stark vermindert (z.B. bei 8-Oxychinolinsulfat) oder erhöht werden kann (z.B. bei TMTD), oder weil völlig neue Bedingungen geschaffen werden können (z.B. HCl-Abspaltung bei Captan).

f. *Wachstumsverlauf.*

Wird die durch die Fungicidwirkung induzierte Wachstumsverminderung der Pilze in Prozent der Kontrolle ausgedrückt, so setzt dieses Verfahren voraus, daß das Pilzwachstum auch unter Fungicideinfluß zeitlich einen linearen Verlauf zeigt. Die Ergebnisse verschiedener Versuche entsprachen der Erwartung und bestätigten die Befunde früherer Autoren (vgl. Batemann [1], Kowalik and Czerwińska [15].

IIB. HAUPTVERSUCHE

Unter den Verfahren zur Laborprüfung von Bodenfungiciden stehen auf der einen Seite die Methoden zur Bestimmung fungistatischer Wirkungen, bei denen alle Stufen bis zum 100%igen Stillstand des Wachstums (fungistatische Grenzkonzentration) verfolgt werden können und auf der anderen Seite die Verfahren zur Ermittlung fungitoxischer Wirkungen, die über die Endwerte der Leistungsfähigkeit eines Fungicids (fungitoxische Grenzkonzentration) Aussagen ermöglichen *.

Es sei erwähnt, daß die Entscheidung zwischen Abtötung und totaler Hemmung mit gewisser Unsicherheit belastet ist, da nach dem Übertragen eines fungicid-exponierten Mycels auf giftfreies Substrat sowohl Versuchsdauer als auch Art des Substrates (Auswaschung, Inaktivierung) das Ergebnis beeinflussen können (vgl. Bateman [1]).

a. *Fungistatische Wirkungen.*

In den Vergleich wurden folgende Verfahren einbezogen:

* Das Verhältnis von fungistatischem zu fungitoxischem Grenzwert ist von der Art des Wirkstoffes sowie von der Reaktionsweise des Pilzes abhängig (McCallan and Wellman [20]).

Agar-Test, Schalen-Test mit Sand oder Torf/Sand-Gemisch, P-Test mit Torf/Sand-Substrat. Jedes Prüfverfahren wurde an drei Fungiciden und drei Bodenpilzen erprobt. Die Ergebnisse sind in Tabelle III zusammengestellt.

TABELLE III

Vergleich verschiedener Verfahren zur Prüfung fungistatischer Wirkungen. ED-Werte in ppm (aktiver Wirkstoff) mit mittlerer Abweichung in Prozent des ED-Wertes.

Fungicid	Verfahren	*Pythium sp.*				*Rhizoctonia solani*				*Fusarium culmorum*			
		ED50	s(%)	ED90	s(%)	ED50	s(%)	ED90	s(%)	ED50	s(%)	ED90	s(%)
Captan	Agar-Test	30	4,7	151	4,7	11	8,5	53	9,6	438	6,2	>5000	—
	S-Test (Sand)	159	8,2	>5000	—	22	4,9	1620	0,9	989	0,8	>5000	—
	S-Test (T/S)	5	7,4	>5000	—	10	4,1	123	9,3	35	7,7	>5000	
	P-Test (T/S)	114	—	175	—	236	10,4	617	10,4	>5000	—	>5000	—
TMTD	Agar-Test	12	14,8	50	—	9	2,0	31	10,5*	18	5,7	307	29
	S-Test (Sand)	10	3,8	1660	5,1	10	7,1	3150	9,1	48	8,0	>5000	—
	S-Test (T/S)	18	0,01	130	1,4	21	7,5	854	2,9	61	2,1	>5000	—
	P-Test (T/S)	68	6,9	141	7,1	113	10,4	278	10,5	688	2,5	1890	2,6
Oxychinolinsulfat	Agar-Test	8	9,0	14	9,3	3	2,6	8	18,0	12	7,6	28	8,6
	S-Test (Sand)	17	19,6	19	19,6	27	12,1	108	12,6	32	15,3	49	15,3
	S-Test (T/S)	65	7,8	101	8,6	62	6,3	153	—	97	20,0	161	20,5
	P-Test (T/S)	217	8,8	284	8,8	267	20,7	300	36,4	357	13,2	515	13,4

S = Schalentest; T/S = Torf/Sand-Gemisch
* Kurve invertiert, Näherungswert

Es steht außer Zweifel, daß die mit Hilfe der Probitanalyse bestimmten ED-Werte eine Genauigkeit aufweisen, wie sie für praktische Belange nicht gefordert werden muß; sie liefern jedoch die Basis für exakte Vergleiche.

Vergleich der Prüfverfahren. Beim Wachstum der Pilze auf begiftetem Agar erhält man, von einigen Ausnahmen zunächst abgesehen, die geringsten ED50- und ED90-Werte. Besonders

ausgeprägt sind die Verhältnisse beim Oxychinolinsulfat. Wird das Agarsubstrat durch Sand ersetzt, dem Nährstoffe in der entsprechenden Konzentration zugesetzt wurden, so wird offenbar der direkte Kontakt des Mycels mit dem Substrat in hohem Maße erschwert, sodaß ein wasserunlöslicher Wirkstoff insbesondere zur Erzielung hoher Hemmprozente eine erheblich geringere Wirkung entfaltet. Nach dem Beimischen von Torf zum Sand (Verhältnis 3 : 7) werden Faktoren wirksam, die in einer Erhöhung des fungiciden Effekts resultieren. Lediglich das Oxychinolinsulfat schließt sich bei allen drei Pilzen dieser Tendenz nicht an. Die Ursachen für diese Erscheinung sind nicht bekannt.

Mit dem P-Test schließlich treten Filterwirkung und Adsorption in den Versuch ein. Das Präparat muß eine etwa 3 cm tiefe Bodenschicht durchdringen, ehe es an den Wirkungsort gelangt. Die Werte liegen infolgedessen in der Regel höher als die entsprechenden Daten aus den Schalentests.

Vergleich ED50 mit ED90: Es ist bemerkenswert, daß bei Captan und TMTD der ED90-Wert um etwa eine bis zwei Zehnerpotenzen über dem ED50-Wert liegt. Aus Abb. 1. kann er-

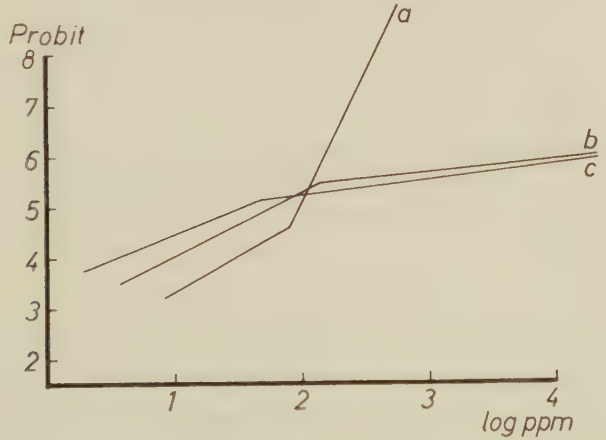

Abb. 1. „Gebrochene" Dosis-Effekt-Kurven bei *Fusarium*-Mycelwachstum auf Torf/Sand-Gemisch mit 8-Oxychinolinsulfat (a), TMTD (b) und Captan (c).
Ordinate: Probit-Werte (= transformierte Prozentwerte).
Abszisse: Logarithmus der ppm Aufwandmenge.

sehen werden, daß diese hohen Differenzen in der „gebrochenen" Wirkungsgeraden eine überzeugende Klärung finden. Bricht die

Regressionsgerade mit steigender Konzentration von einer steilen zu einer flachen Neigung um (Oxychinolin), so liegen die Aufwandmengen für eine 50- bzw. 90%ige Hemmung des Pilzes erwartungsgemäß dicht beieinander. Besonders interessant ist jedoch der umgekehrte Fall, bei dem im niedrigen Konzentrationsbereich der steile Aufstieg erfolgt, und die erzielten Hemmwerte mit der Abflachung der Kurve bis in hohe Konzentrationsbereiche etwa gleichbleiben. Dieser Befund ist wahrscheinlich für die Bewertung von Bodenfungiciden bedeutungsvoll.

Ein Vergleich der Regressionskoeffizienten der ED50- und ED90-„Schenkel" der Wirkungsgeraden zeigt hohe Signifikanz der Differenzen ($P < 0{,}0027$ in allen Fällen).

Vergleich der Pilzempfindlichkeiten: Im allgemeinen gelten Phycomyceten als besonders fungicidempfindlich, Fusarien dagegen als relativ widerstandsfähig. Die vorliegenden Befunde bestätigen diese Auffassung in groben Zügen. Wollte man jedoch daraus schliessen, daß der am schwersten zu bekämpfende Pilz das am besten geeignete Objekt für Fungicidteste sei, so käme man für *Fusarium culmorum* im Agar-, Sand-Schalen- und P-Test zu folgender Reihenfolge der Wirksamkeiten (ED50 sowie ED90): Oxychinolinsulfat vor TMTD vor Captan. Da diese Rangfolge der Wirkstoffe aber weder für *Pythium* noch für *Rhizoctonia* Gültigkeit hat (vgl. Tab. III), halten wir es für notwendig, in jede Prüfung mehrere Pilze einzubeziehen, solange man nicht aufgrund systematischer Versuche und gründlicher Erfahrungen Standard-Testpilze gefunden hat, die eine genügend breite Skala von Reaktionsmöglichkeiten in sich vereinigen.

b. *Fungitoxische Wirkungen.*

In den Vergleich wurden folgende Verfahren einbezogen: Flüssigkeitstest, Schalentest mit Sand und Torf/Sand-Substrat, Z-Test mit Sand, Torf/Sand-Gemisch oder Komposterde. Geprüft wurden auch hier 3 Fungicide und die Empfindlichkeit von 3 Pilzen. Die Ergebnisse enthält Tabelle IV.

Sämtliche Verfahren zur Grenzwertbestimmung haben das gemeinsame Merkmal, daß sie einerseits verhältnismäßig geringen Arbeitsaufwand erfordern, andererseits aber lediglich „Alles-oder-Nichts"-Aussagen erlauben, die zudem vor allem im Bereich der hohen Aufwandmengen mit einer großen Fehlerbreite belastet sind.

Sind wir uns dieser Einschränkungen bewußt, so lassen sich auch hier einige vergleichende Betrachtungen anstellen.

TABELLE IV

Vergleich von verschiedenen Verfahren zur Prüfung fungitoxischer Wirkungen. Grenzkonzentrationen in ppm (aktiver Wirkstoff).

Fungicid	Verfahren	*Pythium* sp.	*Rhizoctonia solani*	*Fusarium culmorum*
Captan	Flüssigkeits-Test	5	50	250
	S-Test (Sand)	>5.000	>5.000	>5.000
	S-Test (T/S)	>5.000	>5.000	>5.000
	Z-Test (Sand)	1.000	1.250	>2.500
	Z-Test (T/S)	250	2.500	>5.000
	Z-Test (Kompost)	>5.000	>5.000	>5.000
TMTD	Flüssigkeits-Test	40	80	400
	S-Test (Sand)	>5.000	>5.000	>5.000
	S-Test (T/S)	>5.000	>5.000	>5.000
	Z-Test (Sand)	800	240	800
	Z-Test (T/S)	160	800	2.800
	Z-Test (Kompost)	>5.000	>5.000	>5.000
Oxychinolin-sulfat	Flüssigkeits-Test	50	25	100
	S-Test (Sand)	60	100	80
	S-Test (T/S)	200	300	150
	Z-Test (Sand)	500	500	200
	Z-Test (T/S)	500	500	1.000
	Z-Test (Kompost)	>5.000	>5.000	>5.000

Vergleich der Prüfverfahren: Der Flüssigkeits-Test wurde in den Vergleich einbezogen, um zu zeigen, daß mit Abweichungen bis zu drei Zehnerpotenzen gegenüber bodenähnlichen Substraten gerechnet werden muß. Sie treten wieder bei hochwirksamen, wasserunlöslichen Fungiciden deutlicher hervor als beim Oxychinolinsulfat. Im Gegensatz zu den oben besprochenen Fungistasis-Verfahren liefern hier die Schalentests bei Captan und TMTD Grenzkonzentrationen, die weit außerhalb sinnvoller Aufwandmengen liegen, sodaß man geneigt sein könnte, diese Wirkstoffe als unbrauchbar auszuschließen. Die Verhältnisse verschieben sich jedoch grundlegend, wenn das abzutötende Inoculum nicht dem begifteten Substrat aufliegt, sondern die Captan- bzw. TMTD-Suspensionen auf dem Wege durch eine Bodenschicht zum Wirkungsort gelangen (Z-Test). Da der Wirkstoff bei diesem Verfahren zweifellos nicht homogen im Substrat verteilt ist, sondern sich durch Adsorption, Filterung und anschließende Einschwemmung wohl Zonen unterschiedlicher Wirkstoffkonzentration herausbilden können, werden

im Gegensatz zu den Schalentests Grenzkonzentrationen unterhalb 5000 ppm erreicht. Diese Effekte sind naturgemäß von der Zusammensetzung des Substrates abhängig. Besonders deutlich wird dies an der Löschung der fungiciden Wirkung bei Verwendung von Komposterde im Z-Test.

Vergleich der Pilzempfindlichkeiten: Im Bereich einer fungitoxischen Grenzkonzentration kommt es zu keinem Pilzwachstum, während bei der (in der Regel) um die Hälfte niedrigeren Konzentration der Pilz noch lebensfähig bleibt. Daher wird ein Präparat treffender als durch den fungitoxischen Endwert durch seinen „Umschlagbereich" gekennzeichnet, der innerhalb dieser beiden Kriterien liegt, und der für bestimmte Pilze eine charakteristische Lage einnehmen kann. Es soll hier zunächst nur festgestellt werden, daß nach globaler Schätzung die Aussagen über die relative Empfindlichkeit der drei Testpilze mit denen aus den Fungistasis-Versuchen übereinstimmen.

III. BESPRECHUNG DER ERGEBNISSE

Der abschließenden Beurteilung der hier geschilderten Methoden zur Labor-Prüfung von Fungiciden sollen einige Überlegungen zu den komplexen Verhältnissen im Boden, soweit sie im Rahmen dieser Untersuchungen wichtig sind, vorangestellt werden.

Der Wirkstoff.

Die Anforderungen an ein brauchbares Bodenfungicid müssen neben der Erfüllung allgemeingültiger Postulate (vgl. Horsfall [11]) um folgende Merkmale erweitert oder abgeändert werden:

1. Der Wirkstoff muß an den Wirkungsort gelangen können, da der Schutz der Pflanze indirekt, nach dem Prinzip der Umweltbehandlung erfolgt. Wasserlöslichkeit ist für Bodenfungicide im Gegensatz zum Blattfungicid nicht grundsätzlich ein disqualifizierendes Merkmal. Hohe Bedeutung gewinnt im Boden die Festigkeit eines Fungicids gegenüber mikrobiellem oder physiko-chemisch bedingtem Abbau.

2. Das Mittel soll im günstigsten Fall ohne Benachteiligung der saprophytischen Bodenflora gegen bestimmte Pathogene gerichtet werden können. Aus dieser Forderung, die für Bodenfungicide nur in dieser allgemeinen Formulierung sinnvoll ist, ergibt sich zwangs-

läufig, daß Verfahren mit großer Breitenwirkung zur totalen Bodensterilisation (Trichlornitromethan, Methylbromid u.a.) hier nicht im Mittelpunkt des Interesses stehen können, da sie in mehrfacher Hinsicht Nachteile aufweisen, die nur in Sonderfällen durch die außerordentlich hohen Abtötungserfolge ausgeglichen werden können.

Der Boden.

Der Boden ist ein Substrat, das durch mannigfache biologische und chemische Prozesse in ständigem Wandel begriffen ist. Durch Fungicideinwirkung darf diese Dynamik, soweit sie biologischer Natur ist, nicht stillgelegt werden.

Darüber hinaus müssen abiotische Reaktionen berücksichtigt werden, bei denen durch Komplexbildung, ungünstige Wasserstoffionenkonzentration oder extreme Redox-Verhältnisse eine chemische Inaktivierung des Wirkstoffes eintreten kann. Schließlich bieten sich bei einer „Umweltbehandlung" durch Aufgießen von Lösungen, Emulsionen und Suspensionen auf den Boden in der Filterwirkung und Adsorption Schwierigkeiten besonderer Art.

Die Wurzel.

Die physiologische Funktion der Wurzel liegt neben den Leistungen im Sekundärstoffwechsel primär in der Wasser- und Nährstoffaufnahme. Zu diesem Zweck wird eine im Verhältnis zur oberirdischen Pflanzenmasse sehr große adsorbierende Oberfläche ausgebildet, deren Empfindlichkeit gegenüber chemischen Agentien ungleich höher ist als die der oberirdischen Pflanzenteile. Das bedeutet, daß die für den Fungicideinsatz wesentliche Spanne zwischen *dosis curativa* und *dosis tolerata* im Bereich der Wurzel relativ gering sein wird. Weiterhin ist zu beachten, daß die Mikroflora der wurzelnahen Zone ein empfindliches Regulativ darstellt, das gleich der echten Mykorrhiza durch chemische Eingriffe in unerwünschter Weise gestört werden kann.

Der parasitische Bodenpilz.

Parasitische Bodenpilze sind nur ein Teil der Mikroflora des Bodens, die in ihrer Gesamtheit auf die Entwicklung der höheren Pflanze wesentlichen Einfluß nimmt. Jeder Eingriff in das Gefüge des mikrobiellen Gleichgewichts führt je nach Art und Stärke der

Bodenbehandlung zu Verschiebungen, deren Dauer von dem Regenerationsvermögen („biocoenotische Pufferung") der Bodenflora abhängig ist, und denen wir durch pflanzenbauliche oder pflanzenschutzliche Maßnahmen eine für die Kulturpflanze erwünschte Richtung zu geben hoffen.

Mit diesen Feststellungen sei angedeutet, um wieviel mehr im Boden das Wirken einzelner Pilze von der Gesamtheit biotischer und abiotischer Faktoren geprägt wird, als das je bei Krankheitserregern an Blatt und Sproß der Fall ist. Zugleich aber ergibt sich aus diesen Überlegungen zwangsläufig, daß ein Prüfverfahren, welches wesentliche Bodenfaktoren einschließt, zu völlig anderen Ergebnissen führen muß als ein weitgehend künstlicher Labor-Routine-Test, in dem sich lediglich relative fungicide Potenzen erfassen lassen.

Bei der Wahl einer geeigneten Prüfmethode wird weiterhin von Bedeutung sein, welches Bekämpfungsziel mit einem bestimmten Wirkstoff erreicht werden soll. So sind zweifellos für die Prüfung radikal wirkender Bodenentseuchungsmittel alle Verfahren geeignet, die zu fungitoxischen Grenzkonzentrationen führen. Kultural anwendbare, also pflanzenverträgliche Wirkstoffe, müssen sehr wahrscheinlich anders beurteilt werden, was sich aus den folgenden Zusammenhängen ergibt:

Die in die Versuche einbezogenen Wirkstoffe Captan und TMTD dürfen als Fungicide mit günstigem chemotherapeutischem Index angesehen werden. Sie sind also zum Schutze wachsender Kulturen geeignet. Aus den Laborversuchen geht jedoch eindeutig hervor, daß sich eine Abtötung von Bodenpilzen mit sinnvollen Aufwandmengen nicht erreichen läßt, denn eine fungitoxische Grenzkonzentration sollte bereits bei etwa 100 ppm (= 0,1 g Wirkstoff pro 1000 ccm Boden = 5.0 g Wirkstoff pro m^2, bei einer Bodentiefe von nur 5 cm und homogener Verteilung, = 2 Liter eines 0,5%igen Fungicidansatzes / Präparat mit 50% Wirkstoff im Gießverfahren / pro m^2) erreicht sein, um hinsichtlich Ausbringungskonzentration und Formulierung innerhalb des technisch Möglichen zu liegen. Da fungitoxische Grenzkonzentrationen in dieser Größenordnung bei Präparaten mit niedrigem chemotherapeutischen Index nicht angetroffen werden, so bleibt für einen Abtötungserfolg der genannten Wirkstoffe kein Spielraum und für den Einsatz ein zu hohes Risiko. Schließen wir in diese Betrachtung die anfangs begründete

Forderung nach weitgehender Schonung der nicht-pathogenen Bodenorganismen ein, so ergibt sich die Notwendigkeit, unter Verzicht auf die Anwendung fungitoxischer Konzentrationen einer partiellen Hemmung des parasitischen Bodenpilzes erhöhte Beachtung zu schenken. Die Versuche über die fungistatischen Wirkungen der Fungicide liefern zu diesen Überlegungen bereits mancherlei ermutigende Hinweise, die im 2. Teil der Untersuchungen weiter verfolgt werden sollen.

IV. ZUSAMMENFASSUNG

1. Aus den besonderen Bedingungen innerhalb des Komplexes „Pathogener Bodenpilz – Substrat – Fungicid" werden einige Grundsätze für die Prüfung von Bodenfungiciden abgeleitet.

2. Die Vorversuche führten zu folgenden Ergebnissen:

a) Sporen sind erheblich empfindlicher als Mycelien,

b) das Alter des Mycels hat innerhalb bestimmter Grenzen keinen wesentlichen Einfluß, während

c) die Art des Impfsubstrates die Fungicidwirkung stark beeinflußt.

3. Sechs verschiedene Prüfmethoden wurden unter Verwendung von 3 Testpilzen (*Pythium* sp., *Rhizoctonia solani*, *Fusarium culmorum*) und 3 Wirkstoffen (8-Oxychinolinsulfat, Captan, TMTD) miteinander verglichen.

4. Die Ergebnisse können bei gleicher Pilz-Fungicid-Kombination bis zu 2 Zehnerpotenzen voneinander abweichen, wenn verschiedene Methoden zur Anwendung kommen.

5. Wegen des häufigen Auftretens „gebrochener" Dosis-Wirkungs-Kurven muß sich die Prüfung über einen weiten Konzentrationsbereich erstrecken. Es ist zweckmäßig, jedes Fungicid an mehreren Testpilzen zu prüfen.

6. Die Hauptergebnisse werden unter dem Gesichtspunkt fungitoxischer und fungistatischer Wirkungen besprochen. Es wird festgestellt, daß die Wirkung der drei geprüften Fungicide bei Anwendung in wachsenden Kulturen und unter erträglichen Aufwandmengen nicht auf einer Abtötung der Mycelien beruhen kann.

7. Es ergibt sich, daß alle Methoden, die die Messung einer partiellen Hemmung des Bodenpilzes erlauben, an Bedeutung gewinnen.

SUMMARY

1. Principles for the assay of soil fungicides are here derived from the special interrelations of the 'pathogenic soil fungus – substratum – fungicide' complex.

2. Preliminary experiments led to the following conclusions:

a) Spores are much more sensitive than mycelium,

b) the age of the mycelium, within certain limits, has no substantial effect on sensitivity,

c) the type of substratum on which the fungus is introduced to the experiment has a considerable influence on fungicidal action.

3. A comparison was made of six different assay procedures, using three test fungi (*Pythium* sp., *Rhizoctonia solani* and *Fusarium culmorum*) and three fungicides (8-quinolinolsulphate, Captan and TMTD).

4. Results given by different assay methods using the same fungus und fungicide could differ by as much as 100-fold.

5. The frequency of 'broken' dose-effect curves makes it essential to test at a wide range of concentrations. Furthermore it is advisable to use several test fungi.

6. The main results are discussed from the point of view of fungistatic and fungitoxic action. It is established that the active substances dealt with here, when applied under cultural conditions in a range of normal quantities, could not exert a direct killing action on fungal mycelium in the soil.

7. All assay methods that allow measurement of partial inhibition of fungal mycelium gain in significance.

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DIE PRÜFUNG VON BODENFUNGICIDEN

II. PILZ – BODEN – WIRT – FUNGICID – KOMBINATIONEN

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EINLEITUNG

Der erste biologische Test zur Beurteilung neu entwickelter fungicider Wirkstoffe ist in der Regel die Kombination von *Pilz* und *Fungicid*, wobei aus den Reaktionen eines oder mehrerer Testpilze auf die mögliche Potenz des Präparates geschlossen wird. Soll die Empfindlichkeit von Mycelstadien geprüft werden, so wird zusätzlich die Aufnahme nährstoffhaltiger *Substrate* in die Test-Kombination erforderlich.

Die letzte Beurteilung erfährt ein Fungicid im Außenversuch unter Verhältnissen der Praxis, wobei die direkte Wirkung eines Präparates auf den Parasiten sich oftmals der direkten Kontrolle entzieht oder auch von nebengeordneter Bedeutung ist. Wichtig ist vielmehr die Summe aller Wirkungsmomente, die sich in dem letztlich erreichten Gesundheitszustand der zu schützenden *Kulturpflanze* äußert.

Zwischen die beiden genannten Prüfungsbedingungen sind jene Testverfahren zu stellen, die unter exakt kontrollierbaren Bedingungen im Labor durchgeführt werden können und dennoch die Reaktionen der Wirtspflanze als Indikator für die fungicide Potenz eines Wirkstoffes einschließen. Es handelt sich also um Pilz-Boden-Wirt-Fungicid-Kombinationen, über die im Folgenden unsere Erfahrungen mitgeteilt werden sollen.

I. MATERIAL UND METHODEN

a. *Pilze*

In die Untersuchungen wurden folgende Pilze einbezogen:

7/54 *Botrytis cinerea*, isoliert von abgestorbenen Pflanzenteilen

36/57 *Pythium* sp., isoliert von Erbsen 1956
37/55 *Pythium* sp., isoliert von Erbsen 1955
38/57 *Pythium* sp., isoliert von Erbsen 1956
40/57 *Pythium* sp., isoliert von Erbsen 1956
6/54 *Rhizoctonia solani*, Herkunft unbekannt
21/54 *Rhizoctonia solani*, isoliert von Erbsen
24/54 *Rhizoctonia solani*, isoliert von Astern 1954
25/54 *Rhizoctonia solani*, isoliert von Kartoffeln 1954
31/55 *Rhizoctonia solani*, isoliert von Begonien 1955

Die Pilze wurden in 300-ccm Erlenmeyer-Kolben mit je 100 ccm Nährlösung (2% Malzextrakt; 0,5% Pepton) bei 20°C vorkultiviert. Von den 10 Tage alten Kulturen wurden die Mycelmassen abgenommen und unter Wasserzusatz im „Starmix" homogenisiert. Die Kulturflüssigkeit wurde verworfen, nur bei den *Botrytis*-Versuchen blieb sie Bestandteil des Homogenisats. Über die Verwendung der Mycelsuspensionen vgl. „Testverfahren". Wird mit total-sterilisierten Böden gearbeitet, so ist es vorteilhafter, *Pythium*-Kulturen nicht zu homogenisieren. Es empfiehlt sich dann die Verwendung von *Pythium*-Agarkulturen zur Bodenverseuchung.

b. *Boden*

Verwendet wurde ein 1 : 1-Gemisch aus Ackererde (sandiger Lehm, Vorfrucht Erbsen) und gedämpfter, mindestens 14 Tage abgelagerter Komposterde (bestehend aus 3 Teilen 3-jähriger Komposterde, 2 Teilen Sand, 1 Teil Torfmull). Diese Mischung ist zweckmäßig, da die Ackererde aufgelockert wird und leichter zu handhaben ist, sie hat jedoch den Nachteil, daß unter Umständen eine *Pythium*-Verseuchung im nicht-sterilen Anteil des Gemisches die zusätzliche *Rhizoctonia*-Verseuchung etwas beeinflussen kann.

c. *Wirtspflanze*

Für die Versuche bewährte sich die anfällige Markerbse „Aldermann". (vgl. Frohberger [3]). Orientierende Versuche mit Mais verliefen weniger befriedigend. Keimprozente der Erbsen in gedämpfter Komposterde 96%. Lagerung des Saatgutvorrates bei 3°C.

d. *Fungicide*

Um Vertreter verschiedener Wirkstoffklassen bei den Versuchen zu berücksichtigen und um klären zu können, ob die Art der Fungicidanwendung (Einarbeitung von Staub in den Boden, Aufgießen von Suspensionen, Emulsionen oder Lösungen) einen Einfluß auf den Bekämpfungserfolg hat, wurden aus einer größeren Anzahl von Wirkstoffen *) die folgenden ausgewählt:

N-Trichlormethylthiotetrahydro-phthalimid
(Captan), aktiver Wirkstoff 50%, Anwendung in wässriger Suspension bzw. in Pulverform.

* Vergleichende Untersuchungen an einer großen Anzahl weiterer Fungicide wurden an anderer Stelle mitgeteilt (Domsch K. H., Die wirking von Bodenfüngiciden I. Wirkstoffspektrum. – Z. Pflanzenkrankh. **65**, 385–405 (1958)).

Pentachlornitrobenzol (PCNB)
Präparat mit einem Gehalt an aktivem Wirkstoff von 25% als Lösung in Xylol (Anwendung als Emulsion) oder Präparat mit 20% aktivem Wirkstoff (Anwendung in Pulverform).

Organische Hg-Verbindung (Methoxyäthyl-Hg-silikat + Phenyl-Hg-acetat), Gesamtgehalt an org. Wirkstoff 2,5%, Anwendung als wässrige Lösung bzw. in Pulverform.

Sofern notwendig, wurden die Fungicide bei Anwendung in Pulverform für niedrige Aufwandmengen mit Talkum gestreckt.

e. *Testverfahren*

Zur Beurteilung der Wirkung von Beizmitteln gegen Bodenpilze sind in den vergangenen Jahren Methoden entwickelt worden, die durch gute Reproduzierbarkeit der Ergebnisse und scharfe Prüfbedingungen unter weitgehend natürlichen Verhältnissen ausgezeichnet sind. Die als „cold-test" oder in einer Abwandlung als "rolled-towel technique" oftmals beschriebenen Verfahren (vgl. Samra [5]) sind bislang nur selten angewandt worden. Es soll daher hier eine kurze Beschreibung des Tests erfolgen:

Prinzip. Durch Verwendung eines nicht sterilen Bodengemisches werden die nicht-pathogenen Mikroorganismen in den Versuch einbezogen; Aufbewahrung bei tiefer Temperatur erlaubt Quellung des Samens und Ausbreitung des Testpilzes, Umstellen auf höhere Temperatur läßt Bekämpfungserfolg in kurzer Zeit sichtbar werden; Etiolement der im Dunkeln aufgestellten Pflanzen wirkt verschärfend auf die Testbedingungen. Zusätzliche Verseuchung des natürlichen Bodens steigert die Gleichmäßigkeit des Befalls und erlaubt eine Differenzierung der Mittelwirkung gegenüber verschiedenen Testpilzen, wobei allerdings der natürliche *Pythium*- bzw. *Rhizoctonia*-Gehalt des Bodengemisches ständig überwacht werden muß, um bei einseitig wirkenden Fungiciden Fehlschlüsse zu vermeiden.

Technik. Einarbeiten der entsprechenden Fungicid-Aufwandmenge in jeweils 7.500 ccm des Bodengemisches. Abfüllen des Boden-Fungicidgemisches in 3 Ton-Pikierschalen zu je 2.500 ccm. Bei Anwendung flüssiger Fungicid-Ansätze Aufgießen von 200 ccm pro gefüllte Schale aus Miniatur-Gießkanne. Aussaat von 8 × 8 Erbsen pro Schale in 2 cm Tiefe am Tage der Fungicidbehandlung des Bodens. Zusätzliche Bodenverseuchung am Tage der Fungicid-Behandlung vor dem Zudecken der Saat. Pro Schale 100 ccm der

Mycelsuspension (s.o.) auf die Saatreihen ausgegossen, was dem Homogenisat einer 10 Tage alten Myceldecke entspricht. Zudecken der Saat, gründliches Anfeuchten. Aufstellen der Schalen 6 Tage lang bei 8°C, dann für weitere 6 Tage bei 25 ± 0.5°C (Dunkel) für *Pythium*, bei 20 ± 0,5°C (Dunkel) für *Rhizoctonia*.

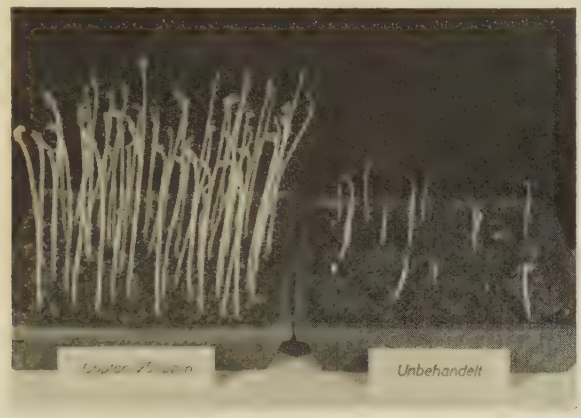

Abb. 1. Cold-Test 12 Tage nach Versuchsbeginn. Erbsen „Aldermann", *Pythium*-Befall.

f. *Auswertung*

Am 12. Tage nach Versuchsbeginn (vergl. Abb. 1) wurden die normal entwickelten gesunden Pflanzen ausgezählt und deren Anteil in Prozent (p') der Gesamtaussaat festgehalten. Die um 4% verminderte Keimfähigkeit der Erbsen in gesundem Boden wurde bei der Beurteilung der Ergebnisse nach der Formel

$$p = \frac{p' - 4}{96} \cdot 100 = 1{,}042\,(p' - 4)$$

berücksichtigt. p ist somit der korrigierte Anteil gesunder Pflanzen an der Gesamtaussaat.

Zwischen der Höhe des Bekämpfungserfolges (% gesunde Pflanzen, Mittelwert aus jeweils 3 Wiederholungen) und der absoluten Streuung der Mittelwerte bestehen interessante Zusammenhänge (vgl. Abb. 2):

1. Einem geringen Bekämpfungserfolg (niedrige Aufwandmengen, ungeeignete Wirkstoffe) ist eine geringe Streuung zugeordnet. Sie beträgt z.B. für den Bereich "0–10% gesunde Pflanzen" im Mittel ± 2,8% gesunde Pflanzen.

2. Die Streuung erreicht bei mittleren Bekämpfungserfolgen hohe Werte von maximal etwa 16%. Der Mittelwert der Streuung im Bereich 51–60 liegt um 7% und damit bei etwa 15% des dazugehörigen Prozentwertes gesunder Pflanzen. Als mittlere Abweichung der Mittelwerte errechnen sich im gleichen Bereich etwa 4% gesunde Pflanzen. Da die überwiegende Mehrzahl aller Versuche Mittelwerte mit geringerer Abweichung liefert, gehört eine hohe Signifikanz der Meßwerte zu den Vorzügen dieses Prüfverfahrens.

3. Im Bereich hoher Bekämpfungserfolge (optimale Aufwandmengen, keine phytotoxischen Schäden, geeignete Wirkstoffe) treten wiederum geringe Streuungswerte auf. Sie ergänzen die Mittelwerte aus Bereichen von

Abb. 2. Beziehungen zwischen Bekämpfungserfolg (% gesunde Pflanzen) und Streuung aus zahlreichen Einzelversuchen zusammengestellt. Die Kurve verbindet die Mittelwerte der Streuung in Klassengruppen zu je 10 Einheiten der Abszisse. Alle Werte über dem Mittelwert tragen das Symbol ⊙.

Abb. 3. *Pythium*-Befall am Hypokotyl der Markerbse "Aldermann". 12 Tage nach der Aussaat.

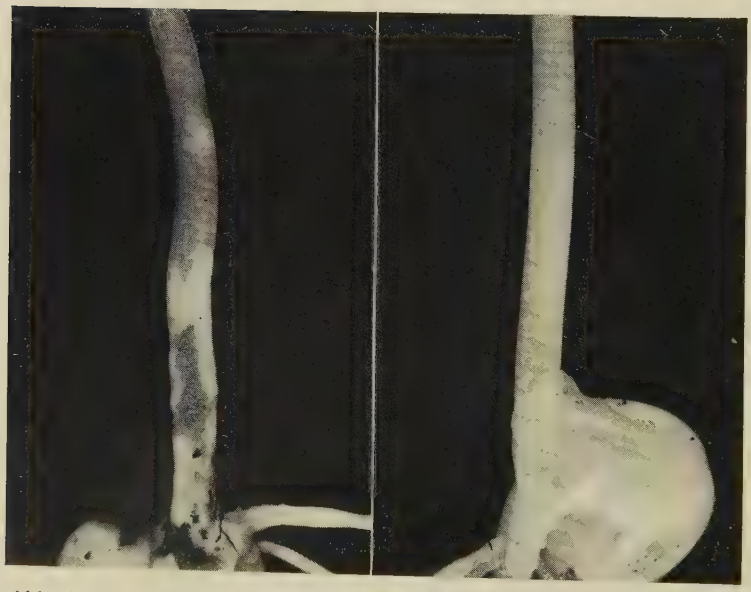

Abb. 4. Links: *Rhizoctonia*-Befall am Hypokotyl der Markerbse "Aldermann". 12 Tage nach der Aussaat. Rechts: Gesunder Keimling.

jeweils 10% zu 10% zu einer Parabel. Z.B. beträgt die Streuung im Bereich "91—100% gesunde Pflanzen" ± 3,0 % gesunde Pflanzen.

Es wird vermutet, daß unter dem dominierenden Einfluß des Parasiten bei schwacher Fungicidwirkung eine Nivellierung der Prozentwerte sichtbar wird, die im entgegengesetzten Falle bei starker Fungicid-Wirkung vom Wirkstoff hei induziert wird.

Im Gegensatz zu reinen Pilz-Fungicid-Kombinationen, bei denen im allgemeinen den mittleren Fungicidkonzentrationen und -wirkungen (Einführung von ED50-Werten, Berücksichtigung von Wägungskoeffizienten) das größere Gewicht zuerkannt wird, liefern Pilz-Fungicid-Wirt-Kombinationen offensichtlich in den extremen Bereichen der Konzentrationsskala besonders wohlbegründete Ergebnisse. Im einzelnen ergab sich bei dieser Art der Versuchsanlage und Befallsbeurteilung in

a) *Pythium*-verseuchten Böden: Samenbefall vor dem Auflaufen sehr häufig (etwa 90% der Erkrankungsfälle), Umfallen nach dem Auflaufen selten. Symptome der erkrankten Pflanzen: stark reduzierter Wuchs oder glasig-wässrige Gewebepartien am Hypokotyl, Kollabieren dieser Gewebe (Abb. 3). Keine Symptome des *Rhizoctonia*-Befalls. Rückisolierungen ergaben eindeutig *Pythium* als Erreger.

b) *Rhizoctonia*-verseuchten Böden: Samenbefall vor Auflaufen weniger häufig (etwa 60% der Erkrankungsfälle), Umfallen nach dem Auflaufem nicht beobachtet. Symptome der erkrankten Pflanzen: rostbraun nekrotisierte Gewebepartien am Hypokotyl, reduzierter Wuchs, teilweise verkrüppelte Primärblätter (Abb. 4), Symptome des *Pythium*-Befalls äußerst selten (0,5% der Erkrankungsfälle) Rückisolierungen ergaben eindeutig *Rhizoctonia* als Erreger.

c) *Botrytis*-verseuchter Boden: Geringer Infektionserfolg des Testpilzes. Rückisolierungen aus erkrankten Pflanzen ergaben zu 60—70% *Pythium*-Befall, der wahrscheinlich auf den *Pythium*-Gehalt der natürlichen Ackererde zurückzuführen ist.

Die erhaltenen Prozentwerte sind exakt reproduzierbar. Sie erlauben die Aufstellung von Dosis-Effekt-Kurven (Abb. 5) sowie deren rechnerische Bearbeitung. Das eigentümliche Phänomen der „gebrochenen" Wirkungsgeraden (Domsch[1]) wurde auch für

den Cold-Test bestätigt. Bei eventuellen Extrapolationen zur Ermittlung von ED95-Werten * ist diese Tatsache zu berücksichtigen. Vorteilhalft ist in jedem Falle eine genügend breite Abstufung der Aufwandmengen innerhalb eines Versuches. Alle Aufwandmengen über dem 95%-Niveau, die zugleich jenseits der Grenze phytotoxischer Schäden liegen bzw. unökonomische Bekämpfungskosten mit sich bringen, scheiden für praktische Belange aus.

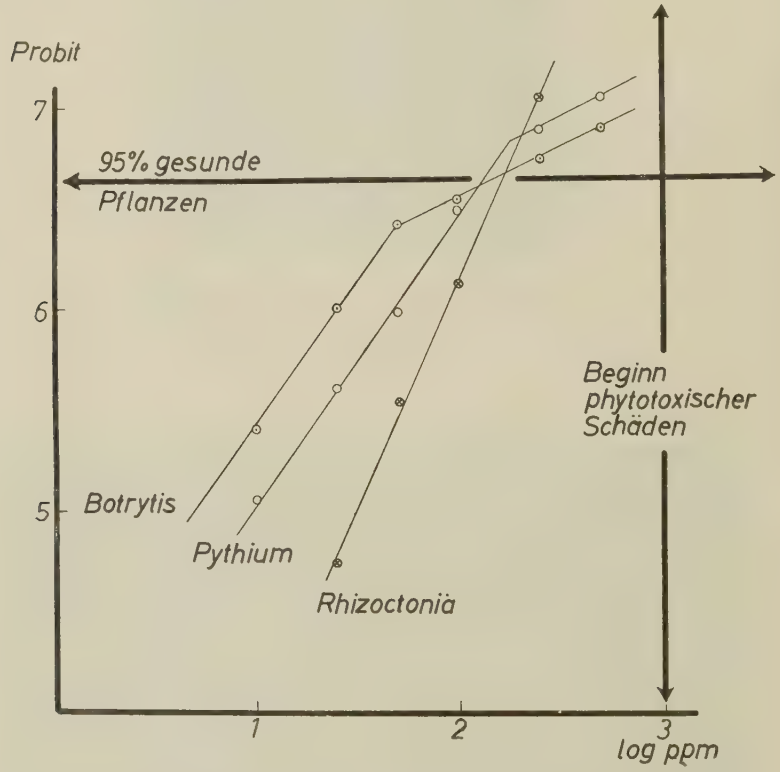

Abb. 5. „Gebrochene" Wirkungsgeraden im Cold-Test. Wirkstoff Captan, 3 verschiedene Testpilze. *Ordinate*: Probit-Werte (= transformierte Prozentwerte), *Abszisse*: Logarithmus der ppm-Aufwandmenge.

IIA. VORVERSUCHE

a) *Verschiedene Pilzstämme*

In Tab. I sind die Ergebnisse aus der Eignungsprüfung von 4 *Pythium*- und 5 *Rhizoctonia*-Stämmen zusammengefaßt.

* Hier: die Dosis, die für einen 95%igen Bekämpfungserfolg erforderlich ist.

TABELLE I

Infektionserfolg verschiedener Pilzstämme im Cold-Test

Pilz	Prozent gesunde Pflanzen
Pythium sp.	

In allen folgenden Versuchen wurde mit dem *Pythium*-Stamm 37 55 und mit dem *Rhizoctonia*-Stamm 31 55 gearbeitet. Die *Pythium*-Stämme sind als gutwüchsig und hochpathogen zu beurteilen. Innerhalb der *Rhizoctonia*-Herkünfte bestehen starke Unterschiede (vgl. auch Flentje und Saksena[4]); der Infektionserfolg ist regelmäßig geringer als in den *Pythium*-Versuchen.

b) *Zeitpunkt der Bodenverseuchung*

Das Einbringen homogenisierter Myzelmassen in den Boden und die unmittelbar vorhergehende oder sofort nachfolgende Fungicidbehandlung der Erde hat große technische Vorteile, aber es läßt sich einwenden, daß sich das Verfahren einem Test mit „freiem Myzelien" nähert, der aus verschiedenen Gründen (vgl. Domsch[1]) Nachteile in sich birgt. Es wurde daher untersucht, in welcher Weise eine vor der Fungizidanwendung zeitlich abgestufte Verseuchung des Bodens den Bekämpfungserfolg beeinflußt. In diesen Versuchen wurde eine entsprechende Fungidmenge in 200 ccm Wasser suspendiert und auf die bereits verseuchten und bei 20°C gelagerten Schalen nach der Aussaat der Samen aufgegossen.

Die Ergebnisse (Tab. II) zeigen für *Rhizoctonia*, daß der Bekämpfungserfolg um so geringer ist, je länger die verseuchte Erde gelagert ist. Der Anteil typischer *Rhizoctonia*-Symptome an den Hypokotylen nimmt im gleichen Sinne ab. Die Ursachen hierfür können in einer verstärkten Bildung fungizid-resistenter Dauerformen sowie in einem erhöhten Befall der Samen zu suchen sein; die Versuche mit *Pythium* zeigen, daß das Infektionspotential mit längerer Lagerung zunächst abnimmt, um später wieder anzusteigen, während sich der Bekämpfungserfolg auf gleicher Höhe hält.

Schlußfolgerung: Das für die folgenden Versuche allgemein gewählte Verfahren der unmittelbaren Aufeinanderfolge von Fungicidbehandlung und Verseuchung des Bodens zeigt für ein gegebenes Fungicid lediglich die Aktionsmöglichkeiten an, die im Augenblick der Verseuchung realisierbar sind. Das Verfahren berücksichtigt nicht die Dynamik, die sich aus der Wiederherstellung von Gleichgewichtszuständen im Boden ("biocoenotische Pufferung") ergibt. Es ist wichtig, sich dieser Einschränkung bei der Beurteilung der Versuchsergebnisse bewußt zu sein.

TABELLE II

Bekämpfungserfolg bei Bodenverseuchung vor Fungicid-Applikation				
Zeitraum zwischen Bodenverseuchung u. Fungicidanwendung (Tage)	Prozent gesunde Pflanzen			
	Rhizoctonia verseucht		*Pythium* verseucht	
	Kontrolle	Captan 500 ppm	Kontrolle	Captan 250 ppm
0	10	99	8	94
5	11	26	24	95
10	19	27	22	92
15	14	9*	15	89
20	5	6*	10	95

* abnehmender Anteil typischer *Rhizoctonia*-Symptome.

c) *Art der Fungicidanwendung*

Bodenfungicide können in den Boden *eingearbeitet* (z.B. in Kombination mit Dünger) oder auf den Boden *aufgegossen* werden (z.B. mit dem Gießwasser im Gewächshaus). Für das Einarbeiten können nahezu alle Wirkstoffe unter vergleichbaren Bedingungen verwendet werden, während Gießmittel als Suspensionen, Emulsionen und Lösungen vorliegen können. Die Ergebnisse einer vergleichenden Untersuchung (Tabelle III) ergeben folgendes Bild:

1. Einarbeitung führt auf der Basis gleicher Bekämpfungserfolge mit dem gleichen Wirkstoff zu einer geringeren Streuung der Meßwerte, d.h. eine homogenere Verteilung des Fungicids im Boden resultiert in einer gleichmäßigeren Wirkung.

2. Einarbeitung führt bei Captan und der organischen Hg-Verbindung zu höheren Bekämpfungserfolgen bei gleicher Aufwandmenge. Das heißt, die beim Aufgießen in Erscheinung tretende Filter- und Adsorptionswirkung der Erde verhindert das Eindringen in tiefere Bodenschichten.

3. Einarbeitung führt bereits bei geringeren Aufwandmengen zu

phytotoxischen Schäden. Das heißt, bei Aufgießen des Fungicids schützen Filter- und Adsorptionswirkung den Wurzelbereich.

TABELLE III

Einfluß des Applikationsverfahrens auf den Bekämpfungserfolg verschiedener Fungicide. Pilz: *Pythium* sp. Angaben in Prozent gesunde Pflanzen. (Mittelwerte aus 3 Versuchen und deren Streuung in % des Mittelwertes).

Aufwandmenge in ppm aktiver Wirkstoff	Captan		PCNB		Org. Hg. Verbindung	
	Staub eingearbeitet	Suspension aufgegossen	Staub eingearbeitet	Emulsion aufgegossen	Staub eingearbeitet	Lösung aufgegossen
0	9	7	11	8	8	10
2,5	—	—	—	—	34 ± 5,6	47 ± 4,1
5	—	—	—	—	43 ± 4,2	52 ± 8,7
10	52 ± 9.0	43 ± 8,1	—	—	—	—
12,5	—	—	—	—	72 ± 4,9	65 ± 11,4
25	73 ± 9,1	51 ± 6,6	9 ± 2,5	26 ± 2,5	80 ± 1,7	72 ± 8,5
50	84 ± 3,8	60 ± 9,0	14 ± 4,2	34 ± 10,8	89 ± 4,1	75 ± 5,0
100	96 ± 1,8	73 ± 10,2	20 ± 7,1	—	*	*
125	—	—	—	49 ± 1,6	*	*
250	97 ± 3,1	76 ± 1,6	23 ± 9,5	54 ± 7,1	*	*
500	98 ± 1,2	78 ± 2,8	*	70 ± 9,0	*	*
1000	*	83 ± 0,4	*	*	*	*
1250	*	95 ± 1,7	*	*	*	*

* = Bereich phytotoxischer Schäden

IIB. HAUPTVERSUCHE

a) *Versuche mit* Pythium *sp.*

Am Beispiel der Captan-Wirkung in *Pythium*-verseuchter Erde sei gezeigt, wie sich die einzelnen Meßwerte eines Versuches zusammensetzen (Tab. IV). Die Ergebnisse lassen erkennen, daß beim

TABELLE IV

Übersicht über die Versuchsdaten aus einem Test mit *Pythium*-verseuchter Erde und Captan (Fungicid eingearbeitet).

Aufwandmenge		Aktiver Wirkstoff (log ppm)	Anzahl gesunde Pflanzen					Probit
Handelspräparat (ppm)	Aktiver Wirkstoff (ppm)		Schale 1	Schale 2	Schale 3	Mittelwert % gesunde Pflanzen		
						p'	p	
2.000	1.000	3,0	57	59	51	87	91	6,34
1.000	500	2,7	60	59	61	94	98	7,05
500	250	2,4	57	61	60	93	97	6,88
200	100	2,0	55	57	59	89	93	6,48
100	50	1,7	53	49	53	81	84	5,99
50	25	1,4	39	50	46	70	73	5,61
20	10	1,0	37	34	26	50	52	5,05
0	—	—	6	7	7	10	10	—

Einarbeiten von Captan im Bereich von 100—500 ppm (aktiver Wirkstoff) ein guter Bekämpfungserfolg zu erzielen ist. Über bzw. unter diesen Aufwandmengen liegt der Bereich erster phytotoxischer Schäden bzw. absinkender Bekämpfungserfolge. Die Abstufung der Aufwandmengen durch annähernde Verdopplung gibt brauchbare Intervalle. Die Schwankungen innerhalb einer Aufwandmenge sind in den jeweils 3 Schalen erstaunlich gering.

Ein anderes Bild ergibt sich bei der Prüfung von PCNB (Emulsion eingearbeitet) und der organischen Hg-Verbindung (Staub eingearbeitet), die in Tab. V noch einmal kurz zusammengefaßt sind.

TABELLE V

Die Wirkung von PCNB und einer organischen Hg-Verbindung in *Pythium*- und *Botrytis*-verseuchter Erde.

Aktiver Wirkstoff (ppm)	Prozent gesunde Pflanzen			
	PCNB		Org. Hg. Verbindung	
	Pythium	*Botrytis*	*Pythium*	*Botrytis*
1250	11	73	—	—
500	35	46	—	—
250	34	29	—	—
125	29	27	29	—
50	12	17	89	87*
25	—	—	80	88
12,5	—	—	72	67
5	—	—	43	—
2,5	—	—	34	—

* etwas gestauchter Pflanzenwuchs

PCNB gibt keinen ausreichenden Bekämpfungserfolg, während mit der Quecksilberverbindung im Bereich sehr geringer Aufwandmengen befriedigende, aber unsichere Effekte erzielt werden. Anstieg und Abfall der Wirkungen erfolgen in einem relativ schmalen Konzentrationsbereich, womit ein erhebliches Risiko in der praktischen Verwendung verbunden ist.

b) *Versuche mit* Rhizoctonia solani.

Die Ergebnisse dieser Versuchsreihe sollen in einer graphischen Darstellung (Abb. 6) veranschaulicht werden. Auf der Ordinatenachse wurden Prozentwerte aufgetragen, die den Probits entsprechen (Wahrscheinlichkeitsordinate). Bei allen Fragestellungen, die über die Bestimmung geeigneter Aufwandmengen hinausgehen,

TABELLE I

Infektionserfolg verschiedener Pilzstämme im Cold-Test

Pilz	Stamm-Nummer	Prozent gesunde Pflanzen
Pythium sp.	38/57	5
	36/57	6
	37/55	7
	40/57	7
Rhizoctonia solani	31/55	11
	24/54	14
	25/54	16
	21/54	35
	6/54	36

In allen folgenden Versuchen wurde mit dem *Pythium*-Stamm 37/55 und mit dem *Rhizoctonia*-Stamm 31/55 gearbeitet. Die *Pythium*-Stämme sind als gleichwertig und hochpathogen zu beurteilen. Innerhalb der *Rhizoctonia*-Herkünfte bestehen starke Unterschiede (vgl. auch Flentje und Saksena [2]); der Infektionserfolg ist regelmäßig geringer als in den *Pythium*-Versuchen.

b) *Zeitpunkt der Bodenverseuchung*

Das Einbringen homogenisierter Mycelmassen in den Boden und die unmittelbar vorhergehende oder sofort nachfolgende Fungicidbehandlung der Erde hat große technische Vorteile, aber es ließe sich einwenden, daß sich das Verfahren einem Test mit „freien Mycelien" nähert, der aus verschiedenen Gründen (vgl. Domsch [1]) Nachteile in sich birgt. Es wurde daher untersucht, in welcher Weise eine vor der Fungicidanwendung zeitlich abgestufte Verseuchung des Bodens den Bekämpfungserfolg beeinflußt. In diesen Versuchen wurde eine entsprechende Fungicidmenge in 200 ccm Wasser suspendiert und auf die bereits verseuchten und bei 20°C gelagerten Schalen nach der Aussaat der Samen aufgegossen.

Die Ergebnisse (Tab. II) zeigen für *Rhizoctonia*, daß der Bekämpfungserfolg um so geringer ist, je länger die verseuchte Erde gelagert ist. Der Anteil typischer *Rhizoctonia*-Symptome an den Hypokotylen nimmt im gleichen Sinne ab. Die Ursachen hierfür können in einer verstärkten Bildung fungicid-resistenter Dauerformen sowie in einem erhöhten Befall der Samen zu suchen sein; die Versuche mit *Pythium* zeigen, daß das Infektionspotential mit längerer Lagerung zunächst abnimmt, um später wieder anzusteigen, während sich der Bekämpfungserfolg auf gleicher Höhe hält.

in der *Botrytis*-Erde als ein gemilderter *Pythium*-Befall erwies. Das heißt, daß eine zusätzliche Verseuchung mit *Botrytis* in nicht-sterilem Boden wirkungslos bleibt. Die für einen Angriff auf die Wirtspflanze günstigen Bedingungen des Cold-Test werden offenbar von anderen Pathogenen früher als von *Botrytis* wahrgenommen.

Die für PCNB und die organische Hg-Verbindung erhaltenen Werte dieser Versuchsreihe decken sich praktisch mit denen aus den *Pythium*-Versuchen (Tab. V).

III. BESPRECHUNG DER ERGEBNISSE

Den beschriebenen Versuchen liegt die Kombination von 4 Partnern zugrunde, die im Laufe eines 12-tägigen Experiments in steter Wechselwirkung stehen. Es entspräche der Erwartung und unseren Erfahrungen z.B. aus Freilandversuchen, wenn wir unter diesen mannigfachen gegenseitigen Einflüssen uneinheitliche Ergebnisse finden würden. Daß das geschilderte Verfahren diese Nachteile nicht aufweist, dürfte folgende Gründe haben:

1. Die Konstanz aller kontrollierbaren Versuchsbedingungen (Bodengemisch, Infektionspotential, Saatgut, Temperatur, Versuchsdauer etc.).

2. Die Dominanz einzelner Partner in entscheidenden Phasen des Versuches (Zusatz-Verseuchung maskiert natürliche Verseuchung, hohe Infektions- bzw. Bekämpfungserfolge werden unter Ausschaltung von Rest- und Nebenwirkungen vom Parasiten bzw. Fungicid diktiert).

Außer diesen günstigen Aussagen über die Versuchstechnik sei ein zweiter Befund von wahrscheinlich nicht geringer Bedeutung hervorgehoben: Mit Captan konnten bei Aufwandmengen von 100–200 ppm gute bis sehr gute Bekämpfungserfolge gegen *Pythium* sp. bzw. *Rhizoctonia solani* erzielt werden. Diese Aufwandmengen liegen weit unter der fungitoxischen Grenzkonzentration in sterilen, bodenähnlichen Substraten (Domsch [1]). Das bedeudet, daß ein hoher Bekämpfungserfolg in einem Konzentrationsbereich erzielt werden konnte, der im Höchstfalle eine etwa 50%ige Hemmung des Pilzes zu induzieren vermag. Wegen des innigen Kontaktes des Pilzhomogenisats mit den Samen ist eine tatsächliche Wachstumshemmung des Pilzes für den Infektionserfolg aber wahrscheinlich

bedeutungslos. So kann zur Erklärung des Phänomens an 2 mögliche Ursachen gedacht werden:

1. Mitwirken der Wirtspflanze durch Einbezug des Fungicids in den Stoffwechsel, dadurch Steigerung der Aktivität. Als Beispiel sei das *in vitro* unwirksame, *in vivo* hervorragend wirkende Chinonoxim-benzoylhydrazon genannt (Frohberger [3]).

2. Mitwirken der Bodenorganismen durch allgemeine antagonistische Einflüsse auf den partiell gehemmten Parasiten unter weitgehender Schonung der Vitalität des Saprophyten. Als Beispiel sei der hinlänglich bekannte *Trichoderma*-Antagonismus genannt (vgl. u.a. Kendrick und Zentmyer [4]).

Der zweiten Hypothese wird in der Mehrzahl der Fälle die größere Wahrscheinlichkeit zukommen. Sie würde – anders formuliert – besagen, daß durch die Einführung eines fungiciden Wirkstoffes in Gaben, die für den Pilz subtoxisch sind, das biologische Gleichgewicht zugunsten der Wirtspflanze verschoben wird. Damit könnte die auf dem Insekticidgebiet seit langem gestellte Forderung nach sinnvoller Synthese von chemischen und biologischen Bekämpfungsverfahren für den Einsatz von Fungiciden erstmalig einer Verwirklichung näher gebracht werden. Über die ausgedehnten Versuche zur Klärung dieser Zusammenhänge wird in weiteren Beiträgen über die Wirkung von Bodenfungiciden berichtet werden.

IV. ZUSAMMENFASSUNG

1. Es werden Versuchsbedingungen für die Prüfung von Bodenfungiciden beschrieben, bei denen die zu schützende Kulturpflanze in die übliche Pilz-Fungicid-Substrat-Kombination aufgenommen wird. Die höhere Pflanze dient zugleich als Indikator für den Bekämpfungserfolg (Cold-Test).

2. Die Eignung verschiedener Pilzstämme (*Pythium* sp., *Rhizoctonia solani*) sowie der am besten geeignete Zeitpunkt für eine Bodenverseuchung mit den genannten Pilzen wird bestimmt. Die Versuchsanlage erlaubt es, spezifische Wirkungen gegen den einen oder anderen Pilz zu erfassen.

3. Die Art der Fungicidanwendung (Einarbeiten oder Aufgießen; Formulierung als Lösung, Emulsion, Suspension oder Staub) hat Einfluß auf den Bekämpfungserfolg sowie auf die Genauigkeit der Versuchsergebnisse.

4. Als Modellfungicide dienen Captan, PCNB sowie eine organische Quecksilberverbindung. Die Ergebnisse sind ausgezeichnet reproduzierbar. Sie können ohne Schwierigkeiten einer statistischen Analyse unterworfen werden.

5. Ein hoher Bekämpfungserfolg wird mit Captan gegen *Pythium* und

Rhizoctonia bei Aufwandmengen erzielt, die weit unter den fungitoxischen Grenzkonzentrationen liegen. Die Konsequenzen, die sich aus diesem Befund ergeben, werden im Sinne einer Synthese zwischen chemischer und biologischer Bekämpfung kurz besprochen.

SUMMARY

1. Experimental conditions for the assay of soil fungicides are described, in which crop plants serve as indicators of the degree of control.

2. The suitability of various types of fungi, and also the most appropriate time for a soil inoculation with *Pythium* sp. and *Rhizoctonia solani*, were determined. The experimental set-up enabled specific action against one or the other fungus to be determined.

3. The method of application of the fungicide (incorporation or drench; formulation as solution, emulsion or suspension) influences the success of the control, as well as the accuracy of the results.

4. As representative fungicides, Captan, PCNB and an organic mercurial compound were used. The results were exceptionally reproducible, and could without difficulty be subjected to a statistical analysis.

5. A high degree of success in control of *Pythium* and *Rhizoctonia* was attained with Captan at application levels considerably below the fungitoxic limit. The implications of this finding are briefly discussed from the viewpoint of a combination of chemical and biological control.

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THE SOLUTION OF γ-MnO_2 BY SUBSTANCES RELEASED FROM SOIL AND FROM THE ROOTS OF OATS AND VETCH IN RELATION TO MANGANESE. AVAILABILITY

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I. INTRODUCTION

It is known that some plants utilize the manganese in soil more readily than others (Samuel and Piper [18], Löhnis [15] and Jones [12]). An obvious reason for this is that plants differ in their ability to absorb soluble manganese. However, since most of the manganese in near neutral soils is insoluble, it is also possible that the differences could be due to the rate of mobilization of the insoluble manganese. Recently it was shown that oat roots release substances which can dissolve manganese oxides (Bromfield [5]). This observation suggested that the differences between plants might be related to the nature and amounts of these substances.

Oat, (*Avena sterilis* L. var. Algerian) and vetch, (*Vicia sativa* L. var. Golden Tares) were compared in the present study since it was found in preliminary experiments that these plants, when grown in a manganese-deficient soil from Penola, South Australia, to which had been added 55 ppm MnO_2, extracted 2 μg and 35 μg manganese respectively after 8 weeks. This result illustrates the pronounced differences which exist between the two plants and agrees with the findings of Jones [12]. The comparison of the two plants included their uptake of manganese from γ-MnO_2 and $MnSO_4$ in sand, the qualitative nature of their dissolving substances, the solution of γ-MnO_2 by these, and the nature of the dissolved manganese.

The fact that plants dissolve γ-MnO_2 raises the question of the relative importance of soil organic matter and root substances in

this process. Leeper[14] and Jones[11] suggest that soil organic matter is largely responsible. In preliminary studies the present author found that cold-water extracts of the air-dried Penola soil contained substances which could dissolve γ-MnO_2. This observation was extended in the present study to assess the importance of these substances in the solution of manganese oxide for plant growth.

II. EXPERIMENTAL PROCEDURE

a. *Pot experiments*

Oats and vetch were grown for eight weeks in 370 g of acid-washed sand contained in 250-ml pots. The pots each received 1 mg Mn as either γ-MnO_2 or $MnSO_4$ and were moistened with 60 ml of modified Arnon's nutrient solution (Bromfield[4]). They were subsequently watered daily to known weight. At about 3-day intervals during the growing period additional 15-ml amounts of nutrient were added to each pot. There were two plants per pot and each treatment was in triplicate. The plants were grown in a glasshouse and at harvest their tops were air dried at 80° C and weighed. Their total manganese contents were determined colorimetrically as permanganate, after acid digestion (Piper[16]).

The manganese-dissolving substances were extracted from the pots by watering them to 100 ml in excess of saturation and decanting the liquid after 6 h. The solution of γ-MnO_2 by these is described later.

b. *Collection of root washings*

For subsequent experiments it was found more convenient to obtain root substances from plants grown in liquid medium. The seeds were pregerminated and 200 of each kind sown onto washed rayon net which was supported on glass rods inside 12 × 10 × 2 in. enamel dishes. Each dish initially received 500 ml manganese-free, modified, Arnon's nutrient solution and was watered from an inverted 1-litre flask via a drip-feed outlet. This maintained the depth of liquid at about $\frac{1}{2}$ in. At weekly intervals an additional 200 ml of nutrient was added to each dish. After 5 weeks the root mats were washed with 50 ml distilled water, and these washings were combined with the medium in the dish. About 600 ml of liquid per dish was collected in this way. It was centrifuged and stored at 4° C. The dry weights of the tops and roots at harvest were of the order of 7.5 g and 2 g respectively for oats and 16 g and 2.5 g for vetch.

c. *Nature of the root washings*

Chromatographic studies. A preliminary purification of the washings was found necessary before chromatographic studies could be made. This consisted of vacuum drying the centrifuged washings (300 ml) then successively extracting the residue, at room temperature, with 2 × 20-ml lots

each of chloroform, 99% aq. ethanol and distilled water. The various extracts were then evaporated to dryness and each redissolved in 15 ml of distilled water. The amounts of γ-MnO_2 dissolved by 2-ml portions of these aqueous solutions are given in Table I. The 70% ethanol extract was by far the most active and there was also some activity in the 99% ethanol and water extracts.

TABLE I

The amounts of γ-MnO_2 dissolved by various fractions obtained by successive extraction of vacuum-dried root washings.

Extract	Mn dissolved * (mg)	
	Oats	Vetch
Chloroform	0.00	0.00
Ethanol 99%	0.035	0.05
Ethanol 70%	0.47	0.25
Water	0.01	0.005
Residue	0.00	0.00
Buffer control	0.00	0.00

* Solution after 1h at pH 5 and 60° C.

These three extracts were examined by descending chromatography in *n*-butanol—glacial acetic acid—water (5 : 1 : 2), (Swain [19]), on 22 × 22-in. sheets of No. 3 Whatman filter paper. The papers were developed by spraying with *M*/1000 aq. potassium permanganate, which resulted in white zones appearing against a light-brown background of manganese oxide. To determine which of the above spots represented substances which could dissolve γ-MnO_2, the extracts were mixed with MnO_2 at pH 4, chromatographed and sprayed with permanganate. The chromatograms no longer contained substances which could decolourize permanganate. This indicated that the substances which decolourized permanganate also react with γ-MnO_2. A more direct but less sensitive method using a colloidal MnO_2 spray (prepared by boiling 100 ml *M*/1000 potassium permanganate with 5 ml glacial acetic acid), was used to positively identify manganese-dissolving substances.

d. *Solution of γ-MnO_2 by root washings*

The ability of the root washings to dissolve γ-MnO_2 was tested by measuring the total amount of soluble manganese as permanganate (Piper [16]) in 20 ml of the filtrate of a mixture of 20 ml root washings, 5 ml water or buffer, 1 ml 0.5% aqueous suspension of γ-MnO_2 and 0.1 ml chloroform, after a measured time interval at 35° C. Acetate, (*M*/5), and borate, (*M*/5), buffers were used when pH control was required. In one experiment the washings were concentrated 6 times and then 2-ml aliquots diluted to give a range of concentrations.

e. *Nature of the dissolved manganese* .

This was studied polarographically and chromatographically.

The manganous ion present in mixtures of root washings and γ-MnO_2 or $MnSO_4$ was determined polarographically using a Cambridge Polarograph. Measurements were made on a solution that consisted of 1 ml each of 0.05% gelatin, 2*N* KCl, test mixture and water, that had been thoroughly gassed with nitrogen. This system was modified slightly for various controls.

The chromatographic studies were made on mixtures which consisted of 0.1 ml each of concentrated root washings (70% ethanol extract), glacial acetic acid and 0.5 mg Mn as manganous chloride solution or γ-MnO_2 suspension. These mixtures, and root washings and manganese sources alone were incubated at 50° C for 48 h after which samples were chromatographed in *n*-butanol—acetic acid—water (6 : 2 : 1). The papers were sprayed with ammonical silver nitrate which caused the spots containing manganese to turn black. Preliminary studies using manganese sources labelled with radioactive Mn^{54} had shown that the spots which turned black with silver nitrate were the same as those which darkened x-ray film and were thus due to manganese. Silver nitrate also produced some additional weak spots. These however could be readily distinguished from those due to manganese.

The various manganese-containing fractions were eluted from the paper and rechromatographed in *n*-butanol—acetic acid—water, and in *n*-butanol saturated with 1*N* HCl (Lederer and Lederer [13]).

III. RESULTS AND DISCUSSION

a. *Stability of MnO_2-dissolving substances in Penola soil*

Before comparing oats and vetch an attempt was made to assess the importance of the solution of γ-MnO_2 by the water-soluble substances observed to be present in air-dried Penola soil. These substances were found to decompose rapidly during moist incubation of the soil. For example water extracts of samples of Penola soil which had been incubated either dry or moist dissolved 0.08 mg and no manganese, respectively, at pH 5 after 24 h at 35° C. The water extract from the dry sample lost its activity on further incubation. This could be prevented by adding, to the extract, a germicide such as chloroform. In view of their rapid decomposition it appears unlikely that these substances contribute very much to the supply of soluble manganese for plant growth.

b. *Studies with plants grown in sand*

(i) Availability of γ-MnO_2 and $MnSO_4$ to oats and vetch. The results of the comparison of the ability of the two

plants to dissolve and absorb manganese from these forms of manganese, are presented in Table II. Both oats and vetch readily utilized the manganese from each source, but they absorbed less from γ-MnO_2. This suggests that the availability of γ-MnO_2 may have been limited by its rate of solution or possibly by the availability of the dissolved manganese itself. However the results do not reveal large enough differences between the two plants to explain the superiority of vetch over oats in Penola soil.

TABLE II

A comparison of yield and manganese contents of oats and vetch grown in sand containing either $MnSO_4$ or γ-MnO_2.
Plants grown for 8 weeks, sown 25.III.1956.

Addition	Oats			Vetch		
	Yield g/pot	Mn-content μg/pot	(log)	Yield g/pot	Mn-content μg/pot	(log)
MnO_2 (1000 μg Mn)	1.57	93	(1.97)	1.15	130	(2.11)
$MnSO_4$ (1000 μg Mn)	1.64	373	(2.57)	1.15	333	(2.52)
NIL	1.61	5	(0.70)	1.10	7	(0.85)
L.S.D. 5%	N.S.		(0.13)	N.S.		(0.17)
1%			(0.26)			(0.20)

(ii) Solution of γ-MnO_2 at pH 5 by washings from sand cultures. The amounts of oxide dissolved by washings of the sand and roots from the previous experiment were 5000 μg and 3700 μg manganese per pot for oats and vetch respectively. This result indicated that there were still appreciable amounts of dissolving substances in the pot cultures, and this suggests that the availability of γ-MnO_2 was probably not limited by them.

The above two experiments show that potentially oats can dissolve and absorb at least as much manganese oxide as vetch. That oats grown in Penola soil can obtain only traces of manganese from γ-MnO_2, whilst vetch obtains appreciable amounts, indicates that there is some factor in the soil which either prevents the solution of γ-MnO_2 by oats or competes with them for the dissolved manganese.

c. *Examination of root washings from plants grown in liquid culture*

Since it appeared that the plant manganese-dissolving substances were important in affecting the availability of γ-MnO_2 in sand, a

comparative study was made of them to determine whether significant differences existed which might account for the superiority of vetch in Penola soil.

(i) Stability of the dissolving substances. The root washings of both plants rapidly lost their capacity to dissolve manganese unless a germicide was present. However, since these substances can be detected in unsterile plant cultures, it appears that they are being produced at a rate which exceeds that of their decomposition by micro-organisms.

(ii) pH and buffer capacity of the root washings. Since the availability of manganese is greatly affected by pH these two measurements were made to assess whether the pH of the rhizosphere of one plant might be more acid than the other. Although the measurements were made on root washings it is likely that they reflect the condition at the root surface. The pH of root washings of vetch was 5.1, of oats 7.1, and of the nutrient solution alone 5.0. Buffer curves were linear over the pH range 4 to 7 for both plants, but 100 ml of oat washings required 11 ml of 0.01 *N* HCl for this change whereas the same volume of vetch washings required only 2.5 ml. It is not known whether the observed differences in pH of the two plants are due to excreted substances or due simply to differential nutrient absorption from the liquid medium.

(iii) Nature of the dissolving substances in root washings. A chromatographic study of the washings was made to determine whether the dissolving substances in them differed qualitatively; such differences might account for the differences between vetch and oats. The chromatograms of the extracts were different for the two plants. A diagram of the chromatogram of the 70%-ethanol extract is shown in Fig. 1. Chromatograms of the 99%-ethanol and water extracts were weak, but the spots which could be detected did not differ from those on the 70%-ethanol chromatograms. The permanganate spray revealed five white spots for each plant but only two of these were common. The strongest and most readily detected spots for oats had R_f values of 0.24 and 0.45 and for vetch 0.15 and 0.48. These strong spots, except that at 0.48 for vetch, also appeared when the papers were sprayed with colloidal MnO_2 which established that oats and vetch release different substances capable of dissolving manganese oxide. No serious attempt has been made to identify

these substances but they appear to react with diazotized sulphanilic acid which suggests that they might be phenolic (Block [2]).

Fig. 1. A diagram of a chromatogram of oat and vetch root washings showing spots which decolourize aq. potassium permanganate and reduce colloidal MnO_2 (shaded spots). (Solvent, *n*-butanol—glacial acetic acid—water, 6 : 1 : 2).

d. *Solution of γ-MnO_2 by root washings*

Since vetch and oats release different dissolving substances a study was made to determine whether the solution of MnO_2 by them was also different.

(i) Effect of pH. The amounts of manganese dissolved over the pH range 4 to 8 by the extracts of each plant are shown in

Fig. 2. With both plants, the amount of manganese dissolved was greatest under acid conditions and rapidly decreased to trace amounts near and above neutrality. Surprisingly the washings from the oat plants dissolved more manganese than did those from vetch plants. This suggested that, other factors being equal, oats might dissolve more manganese oxide than vetch in Penola soil.

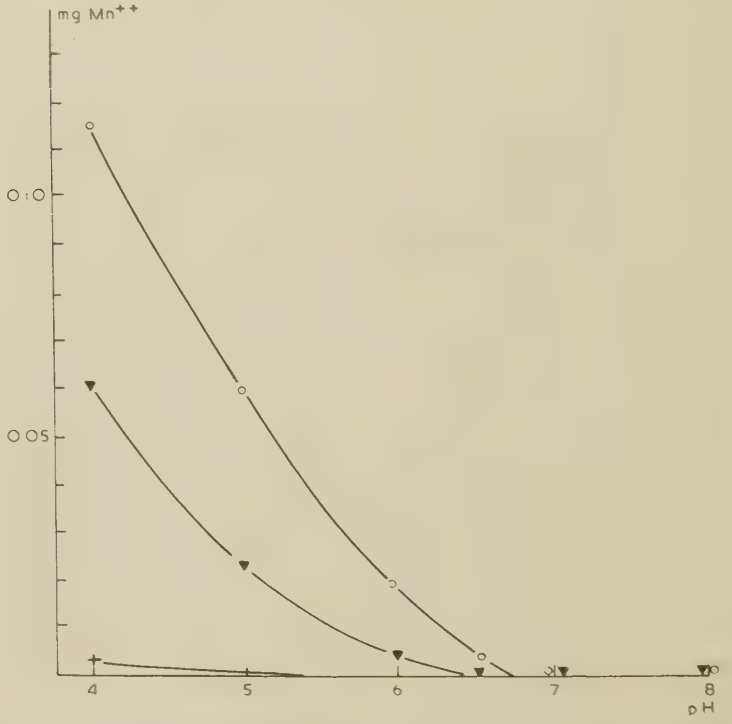

Fig. 2. Effect of pH on the solution of γ-MnO_2 by oat and vetch root washings, showing the amounts of manganese dissolved after 60 h at 35°C.
○ — ○ Oats. ▼ — ▼ Vetch. + — + Control.

(ii) Effect of concentration. One factor which might be different was the concentration of the dissolving substances in the rhizosphere of the two plants. The oat-root system consisted of numerous roots bearing fine lateral roots. These formed a dense mat in the nutrient medium. The vetch-root system on the other hand consisted of fewer lateral roots which were thicker and arose from one main tap root. It was estimated that the length of oat

root, excluding root hairs, per unit fresh weight of root mat was about 2.5 times greater than that of vetch. It seemed possible therefore that the concentration of dissolving substances at any point along the shorter thicker vetch roots could be higher than along the longer thinner oat roots.

The effect of the concentration of the root washings on the solution of γ-MnO_2 was therefore studied and the results are presented in Table III.

TABLE III

Effect of concentration of root washings on solution of MnO_2.

Relative concentrations **	Mn dissolved (mg) *	
	Oats	Vetch
100	.080	.060
80	.075	.040
66	.070	.027
57	.060	.025
44	.045	.015
36	.035	.016
31	.024	.009
26	.007	.015
23	.005	.002

* Solution after 24 h at pH 6 and 35°C

** see text

e. *Nature of the dissolved manganese*

It was possible that vetch and oats dissolved manganese oxide in different ways, perhaps by the formation of organic complexes or by reduction. The different capacities of the two plants to absorb manganese from Penola soil might then be due to the different availability of these forms of manganese. A study was made to determine the forms of soluble manganese present when γ-MnO_2 was dissolved by root washings and when manganous sulphate was mixed with them.

(i) Polarographic studies. The mixtures were examined polarographically in an attempt to detect the presence of manganous ion or other possible forms of manganese. Only manganous ion could be detected in mixtures of root washings of both plants containing either γ-MnO_2 or manganous sulphate. The amounts of manganous ion in mixtures at pH 5 and 7 were estimated to be 0.630 mg and 0.028 mg respectively for oats and 0.650 mg and

0.027 mg respectively for vetch. The total soluble-manganese contents determined colorimetrically, in the pH-7 samples were 0.034 mg and 0.037 mg for oats and vetch respectively. These results suggested that the root washings of both plants reduced γ-MnO_2 to manganous ion and that this form accounted for the greater part of the manganese dissolved at pH 7. It is not known whether the difference between total-manganese and manganous-ion determinations are significant.

(ii) Chromatographic studies. Chromatograms of the various mixtures which were run in butanol—acetic acid—water (Fig. 3) show that they apparently contained different forms of manganese. The various forms which were eluted and rechromato-

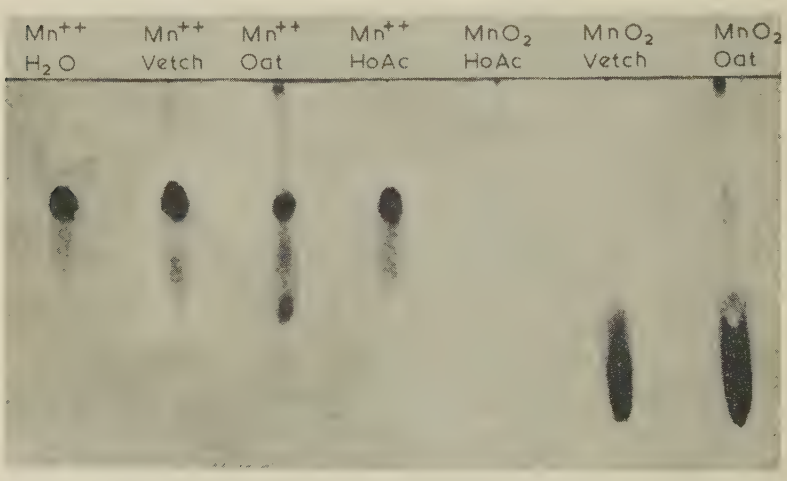

Fig. 3. Chromatograms of mixtures of root washings and manganese sources showing different forms of manganese in the various mixtures. (Solvent, *n*-butanol—glacial acetic acid—water, 6 : 1 : 2; spray, ammoniacal silver nitrate).

graphed in butanol—acetic acid—water ran as two spots. In spite of this, differences still existed, since those fractions from mixtures containing manganous chloride had a strong spot at R_f 0.15 and a weaker one at R_f 0.27 whilst those from mixtures containing γ-MnO_2 had a weak spot at R_f 0.15 and a strong spot at R_f 0.33.

When the same fractions were run in butanol—HCl, only one spot appeared in all cases, which suggested that either the differences

observed in the previous experiment were artifacts or that the fraction which ran with an R_f value of 0.33 was unstable in strong acid.

(iii) Spot tests on the various manganese fractions. Spot tests showed that all the isolated fractions reduced ammoniacal silver nitrate, they all turned brown with concentrated ammonia and that this brown turned dark blue with benzidine, and that those taken from the position corresponding to R_f 0.33 turned light blue with benzidine. These results were consistent with the suggestion that most of the soluble manganese was present in the manganous state. The reason why the fraction from the position corresponding to R_f 0.33 turned light blue with benzidine might be due either to oxidized manganese or to reducible organic compounds.

IV. GENERAL DISCUSSION

It is curious that two plants which have similar mechanisms for dissolving γ-MnO_2 and apparently similar rates of uptake of soluble manganese should differ so markedly when grown in Penola soil. It suggests that in Penola soil there are factors which either limit the solution of γ-MnO_2 by oats or their uptake of the dissolved manganese.

Before considering these factors it is relevant to mention that, in sand, dissolved manganese is freely diffusible whereas, in Penola soil, recently formed manganous ion would be virtually immobilized by various colloids. Thus, in Penola soil, it is likely that only the γ-MnO_2 dissolved along the roots could be absorbed by them, whereas in sand, all the soluble manganese is potentially available. To understand the factors which limit the availability of γ-MnO_2, it is necessary to consider what might be occurring along the roots of the two plants.

The results of the sand-culture and root-washings experiments indicated that oat and vetch dissolve similar amounts of γ-MnO_2. If it is assumed that they actually dissolve similar quantities in Penola soil then to explain the superiority of vetch it is necessary to postulate that the dissolved manganese changes to another sparingly soluble form before the plants can use it. The availability of this second form of manganese would then have to be greater for vetch than for oats. This second form could be similar to the

insoluble organic-manganese complexes suggested by Heintze and Mann [7], Hemstock and Low [8] and Beckwith [1] or it could possibly be manganese tightly absorbed on colloids.

It is possible that the results of the sand-culture and root-washings experiments are misleading and that in Penola soil vetch actually dissolves more γ-MnO_2 than oats. This could occur if vetch had a higher concentration of dissolving substances or hydrogen ions along its roots than oats. Availabilities may then be governed simply by the absolute amount of γ-MnO_2 dissolved at the root surface.

In the above considerations the reduction of γ-MnO_2 by soil organic matter has been omitted. Its contribution might be important if one plant were able to bring about a change in pH. However, the observation that water-soluble, MnO_2-dissolving substances present in air-dried soil are rapidly destroyed during moist incubation suggested that the amount of γ-MnO_2 dissolved in Penola soil by this material would probably be small. Thus it appears likely that the solution of γ-MnO_2 is due mainly to substances released from the plants. This is probably the source of the "influential manganese" referred to by Jones [11].

The manganese in solution appears to be largely manganous, which indicates that solution is due to reduction. There is some indication that the manganous ion then forms a complex. The complex only appeared in mixtures containing γ-MnO_2 which suggests that the complex-forming substance may arise during the reduction of the MnO_2.

It is known that air-drying a soil, or adding antiseptics to it, often increases the amount of manganese which can be chemically extracted from it (Boken [3], Zende [21], Jones [9]). Certain antiseptics can also cure manganese deficiency (Gerretsen [6], Timonin [20]). These various results have been explained by postulating that the treatments inhibit the biological oxidation of manganous ion, and so permit it to accumulate as a result of the reduction of manganese oxides by soil organic matter. It seems from the present work that, in addition, these treatments may also prevent the decomposition of the reducing substances in the soil.

The demonstration that plant roots release compounds which readily dissolve MnO_2 raises the possibility that other insoluble

plant nutrients are also directly dissolved. It is obvious from the present and other work (Rovira [17]) that there are numerous unidentified compounds released from roots which might be active in doing this.

It appears that the different abilities of plants to obtain manganese from γ-MnO_2 in Penola soil maybe related to the availability of some form of insoluble manganous complex formed in the soil or due simply to the different amounts of oxide actually dissolved by the roots. It does not appear to be due to different forms of soluble manganese or to different abilities to absorb soluble manganese.

The marked solution of γ-MnO_2 at acid pH values by root washings probably accounts for much of the increase in availability of manganese to plants which follows the acidification of soils.

V. SUMMARY

Water-soluble substances capable of dissolving γ-MnO_2 were readily extracted from an air-dried, manganese-deficient soil but they were not extracted from it after it had been moistened and incubated.

Availability studies in pots showed that both oats and vetch utilized γ-MnO_2 with ease when grown in sand.

The roots of both plants released substances which dissolved γ-MnO_2. These substances were readily decomposed by micro-organisms; they became more effective as the pH fell below 7 and also as their concentration increased. The substances were different for each plant although the process of solution appeared similar.

Polarograms of the dissolved manganese in mixtures of root washings and γ-MnO_2 were similar to that of manganous ion. Chromatographically, however, it did not behave like manganous ion.

The availability of manganese to plants is discussed in relation to these findings.

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UPTAKE OF PHOSPHORUS FROM P^{32}-LABELLED SUPERPHOSPHATE BY FIELD CROPS

PART. II. COMPARISON OF PLACED AND BROADCAST APPLICATIONS TO BARLEY

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INTRODUCTION

Experiments described in a previous paper (Mattingly and Widdowson [8]) showed that broadcast applications of superphosphate or dicalcium phosphate did not significantly affect the uptake of phosphorus by barley from a small dressing (5 lb. P per acre) of radioactive superphosphate drilled in contact with the seed. No measurements were made of phosphorus uptake from broadcast dressings of radioactive superphosphate. The three experiments described here were carried out at Rothamsted between 1953 and 1955. In 1953 and 1954 placed and broadcast dressings of P^{32}-labelled superphosphate were each tested at one level of manuring; in 1955 each method of application was compared at two rates of dressing. The object of these field experiments was to measure the uptake of phosphorus from placed and broadcast radioactive superphosphate at different stages of growth. In addition, the effects of drilled applications of non-radioactive superphosphate upon the uptakes of broadcast radioactive superphosphate were also examined. The effects of radiation from P^{32}-labelled superphosphate were measured in the 1954 and 1955 field experiments and in a pot experiment carried out in 1954.

Phosphate fertilizers may increase the uptake of *soil* phosphorus by crops in both pot and field experiments. The factors which influence the uptake of soil phosphorus by crops, particularly in greenhouse experiments, were discussed in a recent paper

Mattingly [6], and no literature review is given here. The absolute amount of soil phosphorus that is taken up by field crops in the presence of a phosphate fertilizer has not, however, been studied in any detail. One object of this work was to measure the uptake of soil phosphorus by barley in the presence of broadcast and drilled dressings of superphosphate in order to establish whether the increased yield that usually results from the application of water-soluble phosphates near the seed of cereals could be attributed to greater uptake of soil phosphorus or fertilizer phosphorus or to both.

METHODS AND EXPERIMENTAL DESIGNS

The methods used for determiniúg the total phosphorus content of the crops, and for estimating P^{32} in plant samples have been described in previous papers (Mattingly [7]; Mattingly and Widdowson [8]) and details of the soils from the sites on Highfield have also been given earlier (Mattingly and Widdowson [8]). The experiment in 1955 was carried out on Great-Field, Rothamsted, and the soil, which was slightly acid, had the following analysis:

Ref. No.	pH (water)	% of air-dry soil			P soluble in 0.3*N* HCl (mg per 100 g soil)
		$CaCO_3$	Total P	Clay ($<2\mu$)	
A 9192	5.9	nil	0.064	20.0	0.30

The P^{32}-labelled superphosphate was prepared at the Radiochemical Centre, Amersham, Bucks. Fertilizer applications were based on the total phosphorus content of the superphosphate. The specific activities at the dates of sowing were 0.37, 0.62 and 0.36 mC/g P_2O_5 respectively in 1953, 1954 and 1955.

In 1953 and 1954 both experiments tested the first five treatments below in 5 × 5 Latin Squares:

Treatment	Superphosphate applied (lb. P per acre)			
	Drilled		Broadcast	
	Radioactive	Non-radioactive	Radioactive	Non-radioactive
1	0.0	0.0	0.0	0.0
2	5.0	0.0	0.0	0.0
3	0.0	0.0	10.0	0.0
4	5.0	0.0	0.0	10.0
5	0.0	5.0	10.0	0.0
6	10.0	0.0	0.0	0.0
7	0.0	0.0	20.0	0.0

All seven treatments were tested in 1955 in a 7 × 7 Latin Square. In 1954 and 1955 broadcast or placed dressings of radioactive superphosphate were applied to one half of each whole plot chosen at random. The other half of each plot received an equivalent dressing of non-radioactive superphosphate. Radiation effects were measured by comparing yields and total phosphorus uptakes from adjacent sub-plots.

The size of plots, the number of rows and the variety of barley sown in each year, are shown below:

Variety of barley	Date sown	Spacing (in.)	Length of plot (ft)	No. of active rows per plot	No. of inactive rows per plot
Herta	7-5-53	6	20	9	nil
Herta	14-5-54	8	18	7	7
Haisa II	4-5-55	8	20	7	7

The active and inactive sub-plots in 1954 and 1955 were separated by the width of two rows (16 in.). A basal dressing of ammonium sulphate (2 cwt. per acre) and potassium sulphate (1 cwt. per acre) was broadcast on all plots before sowing. The crops were sampled and harvested in the same way as the previous series of experiments. Two samples were taken before harvest in 1953 and one in 1954 and 1955.

Limitations imposed by the experimental design precluded the use of the one-row seed and fertilizer placement drill, which had proved satisfactory in previous field experiments. Seed furrows were therefore drawn out by hand (using triangular hoes) and the seed and placed dressings of radioactive and non-radioactive superphosphate were sown along the bottom of these furrows. After covering the drilled seed and fertilizer, the broadcast dressings of radioactive or non-radioactive superphosphate were applied by hand and worked into the surface soil with wooden rakes.

RESULTS OF THE EXPERIMENTS

Comparison of broadcast and drilled superphosphate

The effects of broadcast and drilled dressings of superphosphate on yields and total phosphorus uptakes at a sampling four to six weeks after sowing and at harvest are set out in Table 1. Uptakes of phosphorus from P^{32}-labelled superphosphate at different stages of growth in 1953, 1954 and 1955, and the effects from drilling or broadcasting non-radioactive superphosphate are shown graphically on the same scales in Figures 1, 2 and 3.

There were small yield responses to both broadcast and drilled superphosphate at the first sampling in all years. The total phosphorus uptakes were also increased by drilled and broadcast superphosphate; further increases from applying drilled and broadcast

TABLE I

Yields and total phosphorus uptakes (per acre) by barley and effects of drilled and broadcast superphosphate, separately and together.

	Year	Days from sowing	No phosphate	Increase from drilled superphosphate (5 lb. P per acre)		Increase from broadcast superphosphate (10 lb. P per acre)		S. E. of increase
				No broadcast phosphate	Further increase from broadcasting superphosphate (10 lb. P per acre)	No drilled phosphate	Further increase from drilling superphosphate (5 lb. P per acre)	
Early sample								
Dry weights (cwt.)	1953	26	3.0	+1.2**	—0.6	+1.1*	—0.6	±0.37
	1954	40	4.2	+0.4	+0.7	+0.5	+0.5	±0.42
	1955	50	3.9	+2.4**	+0.9	+1.6*	+2.2**	±0.60
P uptake (lb.)	1953	26	1.06	+0.88**	—0.22	+0.69**	+0.04	±0.197
	1954	40	1.87	+0.24	+0.32	+0.28	+0.35	±0.228
	1955	50	1.26	+0.58**	+0.38	+0.55*	+0.59**	±0.207
Harvest								
Dry weights, grain (cwt.)	1953	110	18.7	+0.3	+1.2	+0.5	—0.2	±1.12
	1954	123	19.2	+1.0	—0.2	+0.9	+1.1	±1.12
	1955	107	22.2	+4.8**	+1.6	+2.6*	+3.5**	±1.03
Dry weights, straw (cwt.)	1953	110	24.5	—1.4	+0.9	—1.8	—1.5	±1.16
	1954	123	38.0	—0.1	—0.4	+1.1	+0.9	±2.17
	1955	107	28.6	+4.0**	+0.1	+5.0**	+1.0	±1.05
P uptake, straw + grain (lb.)	1953	110	13.1	—0.6	+0.7	—0.8	—0.2	±0.52
	1954	123	15.4	+0.4	+0.2	+1.4	—0.4	±1.25
	1955	107	7.0	+1.2**	+0.3	+0.9**	+0.6	±0.30

(Significant effects marked ** for $P < 0.01$; * for $P = 0.05$ to 0.01)

dressings simultaneously were, however, small. Yields of grain were only increased significantly in 1955. In contrast to the small effects of the broadcast or drilled superphosphate on yields and total phosphorus uptakes, the amounts of labelled phosphate that were taken up varied with the method of application and with the year in which the experiments were carried out. In 1953, barley grew

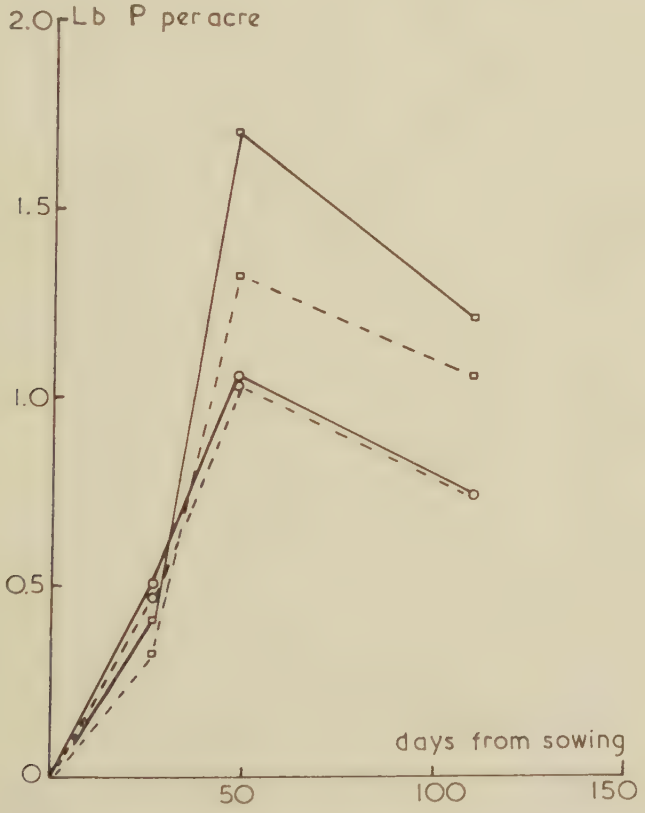

Fig. 1. Uptake of fertilizer phosphorus by barley from broadcast and drilled P^{32}-labelled superphosphate at different stages of growth on Highfield in 1953.

○———○—— 5 lb. P per acre, drilled

- - -○- - -○- - - 5 lb. P per acre, drilled + 10 lb. P per acre as broadcast non-radioactive superphosphate

—— □——— □—— 10 lb. P per acre, broadcast

- - -□- - -□- - - 10 lb. P per acre, broadcast + 5 lb. P per acre as drilled non-radioactive superphosphate.

very rapidly and 26 days after sowing had produced 3 to 4 cwt. of dry matter per acre. The initial rate of uptake of fertilizer phosphorus from both drilled and broadcast superphosphate was rapid (Figure 1). Approximately the same total amounts of drilled and broadcast superphosphate (0.5 and 0.4 lb. P per acre) were taken up during this period but the percentage utilization of broadcast superphosphate (4%) was less than one-half of that obtained with

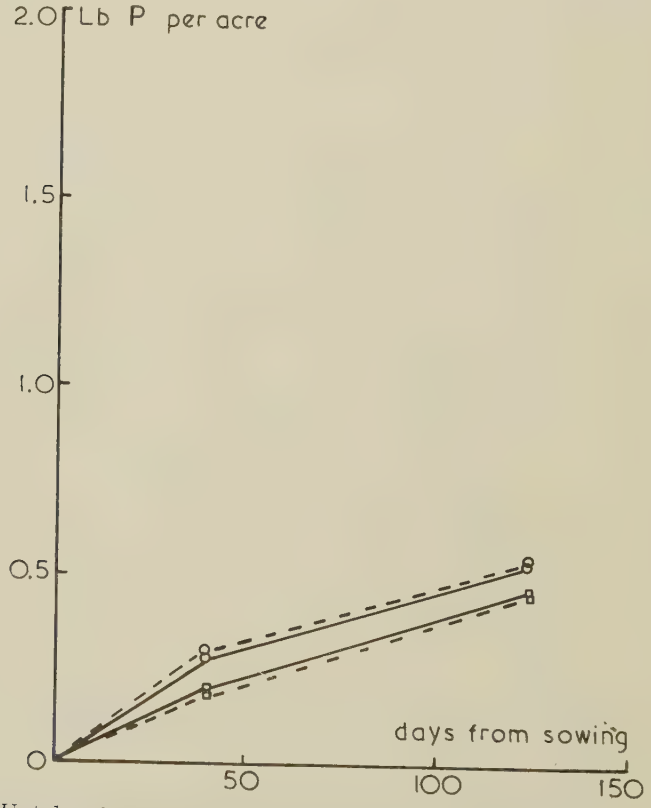

Fig. 2. Uptake of fertilizer phosphorus by barley from broadcast and drilled P^{32}-labelled superphosphate at different stages of growth on Highfield in 1954.

——○——○—— 5 lb. P per acre, drilled

– – –○– – –○– – – 5 lb. P per acre, drilled + 10 lb. P per acre as broadcast non-radioactive superphosphate

——□——□—— 10 lb. P per acre, broadcast

– – –□– – –□– – – 10 lb. P per acre, broadcast + 5 lb. P per acre as drilled non-radioactive superphosphate.

drilled superphosphate. Broadcast superphosphate was also used less efficiently than drilled superphosphate at the second sampling and at harvest. The simultaneous application of non-radioactive superphosphate, either drilled or broadcast, only slightly reduced uptakes of active superphosphate.

Fig. 3. Uptake of fertilizer phosphorus by barley from broadcast and drilled P^{32}-labelled superphosphate at different stages of growth on Great Field in 1955.

- 5 lb. P per acre, drilled
- 10 lb. P per acre drilled
- 5 lb. P per acre, drilled + 10 lb. P per acre as broadcast non-radioactive superphosphate
- 10 lb. P per acre, broadcast
- 20 lb. P per acre, broadcast
- 10 lb. P per acre, broadcast + 5 lb. P per acre as drilled non-radioactive superphosphate.

The rates of uptake of the labelled superphosphate and the total amounts recovered at an early sampling and at harvest were different in 1954 and 1955 from those in 1953. In 1954 both drilled and broadcast superphosphate were taken up slowly (Figure 2) and less phosphate, applied broadcast, was recovered at harvest than from half as much superphosphate drilled with the seed. In 1955, two further rates of application (10 lb. P per acre, drilled and 20 lb. P per acre, broadcast) were tested and direct comparisons were made between the amounts of phosphate taken up from equal dressings of broadcast and drilled P^{32}-labelled superphosphate. The recovery from drilled superphosphate, 50 days from sowing, was about the same (0.5 lb. P per acre) as from twice the amount of broadcast superphosphate. When the rates of application of both drilled and broadcast superphosphate were doubled, the uptake of the fertilizer was increased in the same proportion. At harvest, more phosphate was recovered from the labelled superphosphate in all treatments than after 50 days. As in previous years the effects of applying non-radioactive superphosphate on the uptake of drilled or broadcast radioactive superphosphate were very small (Figure 3).

Radiation effects in the experiments

In 1953 the amounts of phosphate taken up at harvest from plots that received P^{32}-labelled superphosphate were *less* than the uptake from the untreated plots. It was considered possible that these differences resulted from radiation damage as the reduction in total phosphorus uptake was greatest on plots that had absorbed the largest amounts of P^{32}-labelled superphosphate (Table I; Figure 1). In the following two years split-plot designs were used to measure the magnitude of the radiation effects of P^{32}-labelled superphosphate on yield and total phosphorus uptake. These results are set out in Table II.

Radiation may damage apical and root meristems (Mackie, Blume and Hagen [4]) and alter the weight of roots and shoots and the distribution of phosphate in the crop (Martin and Russell [5]). An attempt was consequently made to remove and weigh the roots from radioactive and non-radioactive rows from the experiment in 1954. The roots could not, however, be separated without damaging them seriously as the soil was so heavy in texture.

TABLE II

Yields and total phosphorus uptakes (per acre) by barley from plots receiving non-radioactive superphosphate, and effects from the application of P^{32}-labelled superphosphate.
(Initial specific activity = 0.62 mC/g P_2O_5 in 1954 and 0.36 mC/g P_2O_5 in 1955)

	Year	Non-radioactive superphosphate	Increase with P^{32}-labelled superphosphate	S.E. of increase
a) *Early sample*				
Dry weights (cwt.)	1954	4.9	—0.2	±0.23
	1955	6.6	—0.4	±0.23
P uptake (lb.)	1954	2.23	—0.14	±0.098
	1955	2.04	—0.16	±0.080
b) *Harvest*				
Dry weights, grain (cwt.)	1954	20.0	—0.7	±0.68
	1955	27.2	—0.6	±0.53
Dry weights, straw (cwt.)	1954	38.4	—0.5	±1.04
	1955	33.7	—0.5	±0.58
P uptake, straw + grain (lb.)	1954	7.30	+0.20	±0.245
	1955	8.25	—0.15	±0.140

Root weights were therefore measured in a small pot experiment, using soil from the site on Highfield. Yields and total phosphorus uptakes by barley were compared using samples of the same radioactive and commercial superphosphates that were tested in the field experiment. Differences between treatments, averaging two rates of phosphate (15 and 30 mg P per 800 g soil), are given in Table III.

TABLE III

Yields and total phosphorus uptakes by barley in a pot experiment on Highfield soil, and effects from the application of P^{32}-labelled superphosphate. Results are expressed as the means of two rates of application of superphosphate, 15 and 30 mg P per 800 g soil.
(Initial specific activity = 0.30 mC/g P_2O_5)

	Non-radioactive superphosphate	Increase with P^{32}-labelled superphosphate	S.E. of increase
Dry weights (g)			
Grain	3.44	+0.29	±0.143
Straw	8.88	+0.18	±0.244
Roots	1.36	+0.01	±0.096
Total P (mg)			
Grain	10.8	+0.3	±0.39
Straw	7.6	—0.4	±0.26
Roots	1.5	+0.1	±0.10
Total	19.9	0.0	±0.47

In both years (Table II) yields and total phosphorus uptakes were lower with P^{32}-labelled superphosphate at the first sampling 4–6 weeks after sowing. At harvest, yields of straw and grain were also slightly reduced by radioactive superphosphate. The results show a close agreement in both years in the direction and magnitude of the changes attributed to radiation, with the exception of total phosphorus uptakes at harvest. In the pot experiment none of the effects of P^{32}-labelled superphosphate on growth or phosphorus uptake (Table III) were significant. Radiation did not alter root weights and it had no effect on the total phosphorus uptake in the grain, straw or roots.

Uptake of soil phosphate

The amounts of soil phosphate in the crops at each sampling were calculated from the differences between total phosphate in the crop, determined chemically, and fertilizer phosphate estimated by radioactive assay. Increases and decreases in the quantity of soil phosphate taken up in the presence of drilled and broadcast superphosphate are given in Table IV.

TABLE IV

The increase or decrease in *soil* phosphorus uptakes by barley (lb. P per acre) in the presence of drilled and broadcast superphosphate.

Year	Days from sowing	Drilled superphosphate		Broadcast superphosphate		S.E.
		5 lb. P per acre	10 lb. P per acre	10 lb. P per acre	20 lb. P per acre	
1953	26	+0.38*	—	+0.27	—	±0.134
	48	+1.05	—	+0.39	—	±0.466
	110	—1.23**	—	—1.96**	—	±0.274
1954	40	—0.08	—	+0.24	—	±0.176
	123	—0.49	—	—0.06	—	±0.570
1955	50	+0.08	+0.10	+0.38*	+0.04	±0.152
	107	+0.24	—0.42	—0.21	—0.64*	±0.252

(Significant effects marked ** for $P < 0.01$; * for $P = 0.05$ to 0.01)

Both drilled and broadcast applications of superphosphate *increased* the uptake of soil phosphorus in nine out of the ten comparisons made in the early stages of growth, and in two cases these increases were significant. At harvest however, soil-phosphorus uptake was *reduced* by superphosphate in seven of the eight

available comparisons. Three of these decreases in uptake reached significance. The amounts of *extra* soil phosphate in the crop at two samplings in 1953 that resulted from applying superphosphate are shown in histograms in Figure 4 together with the uptakes of broadcast and drilled superphosphate.

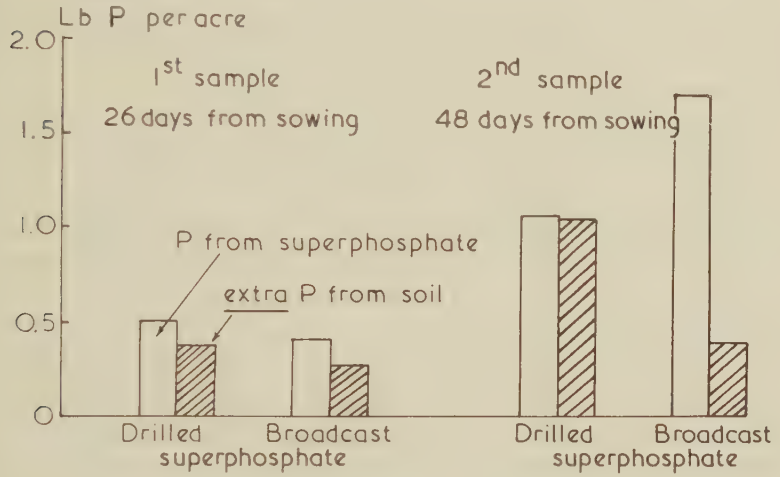

Fig. 4. Uptake of drilled and broadcast superphosphate by barley in 1953 and *increases* in the uptake of soil phosphate 26 and 48 days after sowing.

DISCUSSION

There was no conclusive evidence (Table II and III) from either the field or pot experiments that radiation from P^{32}-labelled superphosphate affected the growth of barley. However, in 1954 and 1955 radioactive superphosphate produced consistently, though not significantly, lower yields and phosphorus uptakes than equivalent non-radioactive dressings (Table II). As a result, detailed comparisons were made between the values for each sub-plot. There was only one significant difference between sub-plots in each year and these results are not presented here. In both years *broadcast* P^{32}-labelled superphosphate produced lower yields and uptakes than non-radioactive superphosphate; no differences were found when drilled applications of the fertilizers were compared. Several workers (Bould *et al.*[1]; Penner [10, 11]) have noticed that the effects of radiation on growth, in both field and greenhouse

experiments, are comparatively sporadic. There was more variation between different years in the uptake of phosphorus from superphosphate when applied broadcast than when drilled with the seed (Figures 1, 2 and 3). It is likely, therefore, that the variations between plots in fertilizer uptake in any year were also greater from broadcast than from drilled dressings; consequently there should be more chance that the amounts of broadcast P^{32}-labelled superphosphate taken up on some sub-plots were occasionally great enough to cause significant changes in yield.

The percentage utilization of superphosphate was higher in 1955 when drilled with the seed than when broadcast, which would be expected as combine-drilled superphosphate usually produces higher cereal yields than broadcasting (Crowther [3]; Prummel[12]). Part of the benefit from both drilled and broadcast superphosphate in the early stages of growth is clearly due to the *extra* quantity of soil phosphate taken up by the crop (Table IV); this was comparable, in 1953, with the amounts of phosphate taken up from superphosphate (Figure 4). There was no evidence in 1954 that barley took up more soil phosphate as a result of broadcasting or drilling superphosphate, than the control plants and in 1955 the extra quantity of phosphate derived from the soil was less than in 1953. These differences in consecutive years are probably related to the rate of growth of the crop; the largest amounts of extra soil phosphate were taken up in 1953 when barley grew rapidly.The data are not sufficiently precise to establish beyond doubt whether drilled superphosphate produced a greater increase in the uptake of soil phosphate than broadcast dressings although there were indications this was so in 1953 (Table IV).

At harvest, however, less soil phosphate was taken up by barley that had received either drilled or broadcast superphosphate than by the control plants; similar results have also been observed in greenhouse experiments with wheat (Spinks *et al.*[13]) and with ryegrass, when grown on soils that did not respond to phosphate fertilizers (Mattingly [7]). Two explanations of these observations are possible. Phosphate ions diffuse away from superphosphate in moist soil and, as a result, progressively more of the soil adsorbs labelled phosphate which is taken up by the plant roots instead of soil phosphate. This simple explanation is supported by the results in Table IV which show that at harvest in 1953 and 1955

the reduction in the uptake of soil phosphate was somewhat greater with broadcast than with drilled superphosphate; moreover, the amount of soil phosphate taken up was less in 1955 when 20 lb. P per acre were applied as broadcast superphosphate than when half this amount was tested. More convincing evidence of the gradual reduction in the quantity of soil phosphate taken up by wheat in the presence of increasing dressings of ammonium phosphate is given by Mitchell [9]. The smaller amounts of soil phosphate in crops receiving superphosphate could also be explained by the alteration in the distribution of roots in the soil which results from broadcasting or drilling superphosphate (Cooke [2]) as the surface area of roots in contact with the soil is likely to be less for plants that have received superphosphate. This explanation would not hold, however, for plants grown in pots which restrict the development of roots.

The labile phosphate ('A' value) in the soil from Highfield in 1953 was estimated from the greenhouse experiment to be 88 ppm P which corresponds to about 176 lb. P per acre; this is about 10 per cent of the total phosphorus in this soil. In the field experiment in 1953 the corresponding value (92 lb. P per acre), determined from the percentage of labelled phosphate in the crop that was derived from broadcast superphosphate, was only half as great. Consequently about half as much soil phosphate, per unit of fertilizer phosphorus, was taken up by barley in the field in 1953 as in the greenhouse experiment in which soil and fertilizer were intimately mixed. This implies that "placement effects" or preferential uptakes of fertilizer phosphorus can occur even on broadcasting, presumably because of the local concentration of superphosphate particles in the top few inches of the soil.

In 1954, when the crop grew very slowly during a cold and wet summer the labile phosphate (340 lb. P per acre) estimated from the amount of P^{32}-labelled phosphate in the barley at harvest was twice as great as in the greenhouse experiment. This high value can only be reasonably explained by the uptake of phosphate from the soil below the layer with which superphosphate was mixed. If it is assumed that the ratio of the uptakes of soil P fertilizer P from the top few inches of soil could not exceed the value (17.6 to 1) found in the greenhouse, the uptake of phosphorus from soil that was mixed with broadcast superphosphate will be $17.6 \times 0.46 =$

= 8.1 lb. P per acre where 0.46 lb. per acre is the amount of broadcast superphosphate in the crop at harvest (Figure 2). As the total amount of soil phosphorus taken up by barley in 1954 (Table I) was 16.3 lb. P per acre, about one half of this (8.2 lb. P per acre) was probably derived from soil below the layer with which the broadcast phosphate was mixed by harrowing. This conclusion supports the findings in other experiments (Mattingly and Widdowson [8]) that there was no advantage in placement over broadcasting in 1954, either on Highfield or on another site low in phosphate, presumably because the uptake of soil phosphate was adequate for maximum yields. These experiments have shown how greatly the amounts of both soil and fertilizer phosphate taken up by barley can differ in consecutive years, even on adjacent sites. The variations in fertilizer uptake in different years are, in fact, as great as those reported by Stanford and Nelson [14] for soils of different phosphate status in the same year.

SUMMARY

1. Three field experiments with barley were carried out in successive years at Rothamsted to compare at different stages of growth, the uptake of phosphorus from P^{32}-labelled superphosphate that was drilled with the seed (5 lb. P per acre) or broadcast on the surface (10 lb. P per acre). Effects of the simultaneous applications of non-radioactive superphosphate were measured in each experiment and different rates of application of drilled superphosphate (5 and 10 lb. P per acre) and broadcast superphosphate (10 lb. and 20 lb. P per acre) were tested in one year. The yields and total phosphorus uptakes obtained with equal amounts of radioactive and non-radioactive superphosphate were compared in two years in the field and in a single greenhouse experiment.

2. P^{32}-labelled superphosphate slightly reduced yields and total phosphorus uptakes in both field experiments but the reductions in yield and uptake were seldom significant. In the greenhouse experiment similar yields and phosphorus uptakes were obtained with both radioactive and with non-radioactive superphosphate.

3. The percentage recovery of phosphate from superphosphate applied in seed furrows was greater at all stages of growth in each year than from superphosphate broadcast on the seed bed. Recoveries at harvest in different years varied less from drilled superphosphate (10% — 15%) than from broadcast superphosphate (5% — 12%). At the rates of application tested in these experiments uptakes of drilled and broadcast superphosphate take place independently throughout the growth of barley.

4. Drilled and broadcast superphosphate both increased the uptake of

soil phosphate by barley during the early stages of growth. In 1953 when the crop grew rapidly, the extra amounts of soil phosphate taken up 26 and 48 days after sowing were equal to 75% and 100% of the amounts of fertilizer phosphorus taken up from drilled superphosphate and 64% and 23% of the amounts of fertilizer phosphorus taken up from broadcast superphosphate. The increased uptakes of soil phosphorus that resulted from applying superphosphate were smaller in 1954 and 1955. In all years at harvest there was less soil phosphate in crops receiving superphosphate than in unmanured plants.

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RESIDUAL EFFECTS OF CALCIUM SULPHATE ON THE YIELD AND COMPOSITION OF A GRASS-CLOVER ASSOCIATION

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INTRODUCTION

The effect of different rates of gypsum on the yield and composition of a grass-clover association in an area of acute sulphur deficiency has been discussed previously in this journal [3]. Only the results for the first year were presented, but because of marked residual effects, the trial was continued for another three years without further additions of gypsum. This paper examines the residual effects on yields of dry matter and nitrogen and the recovery of applied sulphur.

EXPERIMENTAL

Details

A description of the soil, pasture cover and experimental treatments has been presented [3]. Briefly, there were 4 replicates of each of the following treatments: 0, 25, 50, 100 and 200 lb of gypsum per acre. The area was fenced off from stock for the whole period of the trial, and plots harvested when the herbage had grown to a height of 6″ — 9″. Clovers were separated from grass + weeds on individual plots; the two components were dried, weighed, and replicates bulked for chemical analysis.

Rainfall

Rainfall, in inches, at the experimental site, was as follows:

Period	1st Year	2nd Year	3rd Year	4th Year	
Spring–Autumn (about 8 months)	18.8	22.4	39.0	24.3	(Until summer harvest only)
Winter (4 months)	17.5	13.7	14.5	—	

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Most of the growth is made during the first four months and in spite of these relatively high rainfall figures, lack of moisture seriously restricted growth in the first two years, as potential transpiration rates were high. An important figure, if it were available, would be the amount of water leaching through the soil in the first year. An estimate of this is 10 inches.

RESULTS

Yields of dry matter

These are presented in Table I. The variations in total yield of the control treatments reflect the relatively low rainfall figures in the first two years and higher rainfall in the third and fourth years. The highly significant grass responses, amounting to about 1000 lb of dry matter per acre from the heaviest rate of gypsum, were well maintained even in the fourth year. On the other hand, clover responses were only small by the fourth year, and the only significant response, over control, was given by the highest rate of gypsum.

TABLE I

Mean yields of dry matter (lb/acre) from applications of gypsum made in the 1st year.

Gypsum lb/acre	1st Year			2nd Year			3rd Year			4th Year		
	Grass	Clover	Grass + Clover	Grass	Clover	Grass + Clover	Grass	Clover	Grass + Clover	Grass	Clover	Grass + Clover
0	840	40	880	550	20	570	1640	190	1830	2040	200	2240
25	920	200	1120	790	90	880	2070	310	2380	2590	310	2900
50	1230	610	1840	750	360	1110	2350	450	2800	2810	320	3130
100	1410	670	2080	1180	680	1860	2420	580	3000	3550	350	3900
200	1590	710	2300	1620	1230	2850	2540	880	3420	3010	530	3540
Sig. Diff. at 1% Level	Grass 737			Clover 239			Grass + Clover 824					
Sig. Diff. at 5% Level	..	565		..	174		..	615				

Even after 4 years, it is clear that residual effects were still pronounced and it would be necessary to continue the experiment still longer before these effects became negligible. Grasses, in the sulphur-treated plots, were slower in forming seed heads, a darker green, and contained a higher proportion of cocksfoot than the controls. White clover was the dominant legume in the first two years but by the fourth year red clover was the chief legume. This agrees with an observation of P. D. Sears (private communication) that red

clover usually responds less than white clover to sulphur. Perhaps the deeper rooting system of red clover enables it to use sulphate from the lower soil horizons, or it may compete better than white clover with grasses, for sulphate; its more upright habit of growth may also have enabled it to compete better with grasses for light, under the particular cutting management in this experiment.

Nitrogen and sulphur contents of the herbage

The grass and clover were analysed for total sulphur, total nitrogen and in most cases for inorganic sulphur and protein nitrogen. Other analyses were presented earlier. The total sulphur and nitrogen contents are presented in Table II. Gypsum gave marked increases in nitrogen contents of both grass and clover;

TABLE II

Percentages of total nitrogen and sulphur in the dry matter of grass and clover								
Gypsum lb per acre	1st Year		2nd Year		3rd Year		4th Year	
	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover
	% Nitrogen							
0	1.34	2.87	1.06	2.56	1.21	2.54	1.04	2.49
25	1.67	3.11	1.21	2.44	1.35	2.41	0.99	2.44
50	1.64	3.17	1.23	2.58	1.52	2.77	1.12	2.61
100	1.71	3.53	1.42	2.81	1.54	2.84	1.15	2.63
200	1.73	3.45	1.66	2.90	1.65	2.83	1.27	2.59
	% Sulphur							
0	.14	.17	.11	.11	.12	.14	.08	.10
25	.31	.29	.11	.11	.12	.11	.08	.12
50	.35	.42	.12	.12	.13	.15	.08	.12
100	.40	.30	.12	.14	.13	.16	.06	.12
200	.32	.34	.16	.17	.14	.16	.07	.15

the effects, although small by the final cut, were maintained over the whole four years. This increase in per cent nitrogen was almost entirely in protein nitrogen, as the non-protein nitrogen remained very steady at about 0.5 per cent in the grass and 1.0 per cent in the clover, thus confirming similar findings in another experiment [2]. Sulphur contents fell to exceptionally low levels by the fourth year, and the differences between treatments were less. In the first year, inorganic sulphate was considerably less in the clover than in the grass, and clover contained only traces in subsequent years. Inorganic sulphate was 25 per cent of the total sulphur in the grass

in the first year for the 200 lb gypsum treatment, but was considerably less in subsequent years. The N : S ratio in the grass was 4·6 in the first year in the gypsum treatments, probably due to luxury consumption of sulphur; in the second and third years the ratio remained remarkably steady at 10–12, but rose to 12–18 in the last year. Apart from the first year, when the N : S ratio in the treated clovers was 8–12, the ratio in the clovers was about 25.

Yields of nitrogen

These are presented in Table III. A comparison of the last columns of Tables III and IV shows that 8 to 12 lb of nitrogen were yielded for each pound of sulphur recovered. For the total of the four years the extra yields of nitrogen were divided remarkably evenly between grass and clover, but while clover yielded more nitrogen than grass in the first years, clover responses were less than grass responses in the last two years.

TABLE III

Mean yields and yield increases of nitrogen in grass and clover. Pounds per acre.

Gypsum lb per acre	1st Year			2nd Year			3rd Year			4th Year			TOTAL		
	Grass	Clover	Grass + Clover	Grass	Clover	Grass + Clover	Grass	Clover	Grass + Clover	Grass	Clover	Grass + Clover	Grass	Clover	Grass + Clover
0	11	1	12	6	1	7	20	5	25	21	5	26	58	12	70
Yield increase of nitrogen in sulphur-treated plots															
25	4	3	7	4	1	5	8	2	10	5	3	8	21	11	32
50	7	20	27	3	8	11	16	8	24	10	3	13	36	37	75
100	15	21	36	11	18	29	17	11	28	20	4	24	61	56	117
200	17	29	46	21	35	56	22	20	42	17	9	26	77	87	164

Recovery of sulphur

Total uptake of sulphur over the four years by both grass and clover in the control treatments was 5·9 lb per acre (last column, Table IV). Of this total, about 10 per cent (0·6 lb) was taken up by the clover and 90 per cent by the grass. As seen in the last three columns of Table IV, more of the applied sulphur was recovered in the grass than in the clover, but clover recovered a bigger proportion of the sulphur as increased rates of gypsum were applied.

The biggest proportion of the sulphur was recovered in the first year, but even in the fourth year, total recovery of sulphur was

half a pound or more per acre. A comparison of the first and last columns shows that about 80 per cent of sulphur was recovered from the dressings of 25 and 50 lb gypsum per acre, about 50 per cent from the 100 lb dressing and 33 per cent from the 200 lb dressing.

TABLE IV

Recovery of sulphur. Pounds per acre.															
S applied as gypsum lb/acre	1st Year			2nd Year			3rd Year			4th Year			TOTAL		
	Grass	Clover	Grass + Clover	Grass	Clover	Grass + Clover	Grass	Clover	Grass + Clover	Grass	Clover	Grass + Clover	Grass	Clover	Grass + Clover
0	1.1	0.07	1.2	0.6	0.02	0.6	1.9	0.3	2.2	1.7	0.2	1.9	5.3	0.6	5.9
Yield increase of sulphur from treated plots															
5.2	1.8	0.5	2.3	0.3	0.1	0.4	0.5	0.1	0.6	0.5	0.2	0.7	3.1	0.9	4.0
10.5	3.2	2.4	5.6	0.3	0.4	0.7	1.3	0.4	1.7	0.7	0.2	0.9	5.5	3.4	8.9
20.9	4.5	1.9	6.4	0.8	0.9	1.7	1.3	0.7	2.0	0.3	0.2	0.5	6.9	3.7	10.6
41.8	4.0	2.3	6.3	1.9	2.0	3.9	1.8	1.2	3.0	0.5	0.6	1.1	8.2	6.1	14.3

DISCUSSION

Reference has previously been made [2] to the marked residual effects of sulphur applied as gypsum in a dry year. The results presented here provide further evidence of this. As further experiments using S^{35} are under way, to examine the mechanisms whereby sulphur is retained against leaching, it is not intended to discuss this problem in any detail.

The form of the gypsum may be important. Commercial samples of gypsum entering this country from Australia contain much coarse, hard material which should dissolve more slowly than the finely powdered gypsum used in these trials. Rainfall, particularly in the year of application, is obviously important and at the heaviest rates of application large amounts of gypsum were probably lost by leaching even during the relatively dry first year. Unquestionably root reserves of sulphur must serve as an important source in the following season, particularly as the weight of roots produced may amount to at least half the above-ground production [4]. Making this assumption, and also that the roots contain the same percentage of sulphur as the tops, extra root reserves of sulphur in the first year (half the uptake by the tops) would have amounted to 1.2, 2.8, 3.2 and 3.2 lb per acre from the 5.2., 10.5, 20.9 and 41.8 lb of

sulphur treatments respectively. These calculated reserves would account in large measure, except in the case of the heaviest treatment, for the further recovery in the next three years of 1.7, 3.3, 4.2 and 8.0 lb respectively from these treatments.

The increased yields of nitrogen in the grass from sulphur treatments in the first year were shown to be due to underground transfer of nitrogen from clover rather than to the stimulation of mineralisation of soil organic nitrogen [3]. This increase was well maintained over the course of the trial and was relatively more significant than the direct contribution from the clover in the last two years. Butler and Bathurst [1] have recently suggested that the sloughing off and decomposition of nodules could account for the underground transfer of nitrogen from clover to grass; clover-root decomposition must also be an important factor. However, actual excretion of nitrogen compounds should not be dismissed too lightly. If sulphur is deficient, the formation of primary, low-molecular nitrogen compounds may proceed more rapidly than they can be removed to form protein in the plant. This should lead to the accumulation of soluble nitrogenous compounds of low molecular weight in nodule and root tissue, which is a possible prerequisite for excretion to take place. The high nonprotein nitrogen contents of the clovers (about 1 per cent) and their high N/S ratios, particularly after the first year, lend support to this suggestion.

Gypsum at the heaviest rate gave a total increase of 164 lb of nitrogen per acre over the four years, and a corresponding large increase in yield of dry matter. To have obtained the same yields of nitrogen by the use of nitrogen fertilizers may have needed some 300 lb of fertilizer nitrogen (about 50 per cent recovery), or 1500 lb per acre of a fertilizer containing 20% N; furthermore sulphur deficiency is so acute in this area that some sulphur would also be necessary to enable grasses to respond to fertilizer nitrogen [2]. At the present price ratios of nitrogen and sulphur fertilizers there is no question that the principle of the satisfactory establishment and nutrition of suitable legumes is the correct one for these areas.

SUMMARY

Gypsum at rates of 0, 25, 50 and 100 and 200 lb per acre was applied to a grass-legume association, and the effects on the yields of dry matter,

nitrogen and sulphur were followed for four seasons. Pronounced residual effects were noted and had not completely disappeared by the last harvest. Underground transference of nitrogen from clover to grass over the four years was about equal to the nitrogen harvested in the clover. Of the sulphur applied, 80, 80, 50 and 30 per cent were recovered from the 25, 50, 100 and 200 lb rates of gypsum respectively. Eight to twelve pounds of nitrogen were fixed for each pound of sulphur recovered.

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COMPOSTING OF STRAW IN SMALL UNITS

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INTRODUCTION

It is now common practice to plough straw into soils, generally in the autumn, and to add extra nitrogen in the spring to prevent the crop becoming deficient in this element due to fixation of soil nitrogen by the micro-organisms decomposing the straw. This practice is generally carried out to improve the physical condition of the heavier-textured soils although the presence of straw may reduce losses of nitrogen through leaching during the autumn and winter [2].

Crowther [4] has stated "whereas on the farm scale it is not justifiable to buy inorganic nitrogen to rot straw to composts, in horticultural work the physical effects of bulky organic manures are so urgently needed that composting is to be recommended". The main advantages of using composted over fresh straw are (a) even where extra nitrogen is applied with the straw there is a danger of localized deficiency of nitrogen in the crop due to the difficulty of intimately mixing the straw with the soil, (b) composting may help to kill weed seeds and also substances toxic to plants and animals, particularly where animal or human excretions are added to the compost heaps.

The literature on composting has been reviewed in a technical communication [1], and more recently by Hoyle and Mattingly [7].

Studies comparing the effects of straw and composts on soil properties and plant growth and quality were started in this laboratory in 1951. Since it was not feasible, in view of labour difficulties and costs, to prepare composts on a large scale, preliminary work was concerned with the preparation of composts in small units. In 1951 wire units holding 100 lb. straw (dry basis) were used. Because of the unsatisfactory nature of the composts prepared

in this way two large cells were made of breeze blocks, holding 1500 lb. straw, and a number of small cells made of concrete paving slabs, holding 30 lb. straw, were used in later years. Since the composts prepared in both these types of units were equally satisfactory, work in later years was continued in the small cells. This paper will report on the properties of composts prepared in the three types of units, with special reference to dry-matter losses and changes in nitrogen content, since it appears that these factors are satisfactory criteria of the degree of composting. It is interesting to note that Hoyle and Mattingly [6] found that straw could be composted satisfactorily with sewage sludge in brick cells very similar to the small concrete units described in this paper.

EXPERIMENTAL

Composting Units

(1) Wire Units. The material rested on the ground and was retained in 2 in. mesh "chicken" wire cages 3 ft × 3 ft × 2½ ft high. These units held 100 lb. chaffed straw (dry basis).

(2) Large cells. 6 ft × 6 ft × 6 ft internal dimensions (with door) made of breeze blocks lined with cement inside and outside. The concrete floor sloped slightly to one side and drainings ran into a cement-lined sump. These cells held up to 1500 lb. straw.

(3) Small cells. (Constructed from concrete paving slabs 2 ft × 2½ ft × 2 in. thick) having internal dimensions 2 ft × 1⅔ ft × 2½ ft high. The cells were arranged in units of four with a common internal wall and the slabs were held together with steel corner pieces. The cells rested on concrete slabs. The arrangement has the advantage that it may be dismantled easily. Each cell held 30 lb. chaffed straw.

The wire units and large cells were not covered whilst the small cells were covered with ridged canvas to protect them from rainfall and direct sunlight.

Prior to adding the accelerator the straw in the wire units and small cells was wetted by playing a hose on the material for 1–2 hours each day for three days. The heaps were allowed to drain for 24 hours and the appropriate accelerator then mixed in after turning out the heap on to a length of tarpaulin. The material was replaced in the unit and pressed down somewhat. The material in each of these units was mixed after 14 days.

The large concrete cells were filled by placing alternate 1-ft layers of dry straw and sufficient accelerator (usually 'nitro-chalk') to give an initial carbon/nitrogen ratio of 40. Sufficient water was sprayed on to each layer to give an estimated water content of 70%. Each layer was trampled to consolidate it. Considerable drainings ran into the sump, and these were returned periodically to the top of the heap. The material in the large heaps was not turned but after about 4 weeks, when the level had sunk by about 50%, further straw and additions of 'nitro-chalk' and water were made.

No attempts were made to artifically aerate any of the units. The wire units were too well aerated naturally as indicated by high water losses

through evaporation. The large and small cells appeared to be sufficiently well aerated as indicated by the absence of any foul smell at any stage of rotting even at the bottom of the heaps.

Analytical Methods

The whole of the material in the heaps was weighed and samples (4–5 lb. from the small units and 40–50 lb. from the large units) taken for determination of moisture and other constituents. The samples were dried in a forced-air oven at 60° and ground in a mill to pass an 0.5 mm sieve. Total nitrogen was determined by the usual Kjeldahl procedure using salicylic acid and sodium thiosulphate to "fix" nitrate. Organic carbon was determined in the apparatus described by the author [3]. Ammonia and nitrate were determined in 0.5*N* sodium-acetate extracts by distillation and nesslerisation [9] and by phenoldisulphonic acid respectively [5].

RESULTS

Moisture losses through evaporation

At the start of composting the moist straw in the wire units and small concrete cells contained 70 to 73 per cent moisture (wet basis). Moisture losses were relatively low from the small concrete cells and it was only necessary to add small amounts of water at turning and three or four more times to maintain the materials in thoroughly moist condition. Excessive evaporation occurred from the wire units and the heaps dried out to a depth of about 9 inches at the top and sides. This dried portion could not be re-wetted easily, except by applying large volumes of water, most of which ran out at the bottom of the heaps.

In most years the sump drainings and rainfall were sufficient to maintain the material in the large concrete units in moist condition. There was a tendency for the upper 6 to 9 in. of material to dry out somewhat, but this represented only a small portion of the material in the heap.

Dry-matter losses

In their pioneer work on composting of straw Hutchinson and Richards [8] found that about half the initial dry matter was lost if sufficient extra nitrogen was added to give an initial carbon/nitrogen ratio of 40, and that the final material had a carbon/nitrogen ratio near 20.

In these studies the heaps were set up in May and allowed to rot

for 4 to 5 months. Typical results obtained with the three types of units are given in Table I, in which straw was composted with sufficient added 'nitrochalk' to give an initial carbon/nitrogen ratio of 40. It will be seen that the materials in both the small and large concrete units lost between 51 and 59 per cent of their initial dry matter, whilst the materials in the wire units lost only 32 to 35 per cent of their dry matter.

TABLE I

Dry-matter losses (% of initial dry matter) from straw composts receiving sufficient nitro-chalk to give initial C/N of 40 (5 months composting).

Composting units	1951	1952	1953
Large concrete cells . . .	—	51	54
Small concrete cells . . .	—	55	59
Wire units	32	36	35

Dry-matter losses as related to time of composting and temperature of heaps

Table II shows dry matter losses after 14, 79 and 139 days from a straw + 'nitro-chalk' and a straw + grass compost (both having initial carbon/nitrogen ratio of 40) in the small concrete units.

TABLE II

Dry-matter losses (% of initial dry matter) as related to time of rotting. (Small concrete units)

Time (days)	0–14	14–79	79–139
Mean daily temperature of heaps (°C)	37	24	17
Material			
Straw + 'nitro-chalk' (initial C N 40)	19.6	43.3	1.9
Straw + grass (initial C/N 40)	27.3	32.7	3.0

During the first 14 days of composting from 19.6 to 27.3 per cent of the initial dry matter lost. Both heaps showed a very similar time-temperature curve for all periods and during this period their mean daily temperature was 37°C. During the next 65 days of relatively cool rotting (mean daily temperature of the heaps of 24°C) an even greater proportion of the dry matter was lost (32.7 to 43.3 per cent of initial dry matter), whilst during the next 60 days (mean temperature of heaps 17°C) relatively small amounts of dry matter were lost (1.9 to 3.0 per cent of initial).

Tests in other years showed that dry-matter losses in the small concrete units ranged from 48 to 64 per cent of the initial dry matter providing that sufficient readily available nitrogeneous accelerator ('nitro-chalk', grass, ammonium sulphate, urea, dried blood) was added to give an initial carbon nitrogen of not more than 40.

Changes in nitrogen and carbon nitrogen ratios

During the initial trials with the two small types of units the effects of varying the initial carbon nitrogen ratio (by supplying different amounts of 'nitro-chalk') on final carbon nitrogen ratio and nitrogen losses were studied. Results are shown in Table III.

TABLE III

Effect of initial C N on final C N and nitrogen lost after 5 months composting.

Material	Initial C/N	Small concrete units		Wire units	
		Final C/N	N lost as % of initial	Final C/N	N lost as % of initial
Straw alone	145	58.0	4.1	62.1	5.2
Straw + 'nitro-chalk'.	40	20.6	8.0	32.5	40.3
Straw + 'nitro-chalk'.	27	20.8	39.3	34.7	62.6

Where straw was composted without any additional nitrogen the final product had a high carbon nitrogen ratio (58.0 to 62.1). When sufficient nitrogen was added to give an initial carbon nitrogen ratio of 40 or where even more nitrogen was supplied so as to give an initial ratio of 27 the final materials had a final carbon nitrogen ratio very near 20 in the case of the small concrete cells but rather higher in the case of the wire units. Losses of nitrogen were much higher from the wire units than from the concrete cells. The higher level of added nitrogen gave no advantage as far as composting was concerned and merely resulted in loss of the extra nitrogen. Even with the "optimum" amount of initial nitrogen, giving a carbon nitrogen ratio of 40, about 8 per cent of the initial nitrogen was lost. Tests in other years in the small cells using a variety of nitrogen sources so as to give an initial carbon nitrogen ratio of 40, resulted in losses of nitrogen ranging from 7 to 14 per cent.

A few tests in the large concrete units using straw with sufficient

added 'nitro-chalk' to give an initial carbon/nitrogen ratio of 40 showed losses of nitrogen ranging from 3 to 13 per cent.

Changes in temperature

Temperatures near the middle of the heaps were taken with a "maximum" thermometer contained in a pointed metal probe. Typical results for straw-'nitro-chalk' composts (initial carbon/nitrogen ratio 40) in the three types of units are shown in Fig. 1. Smooth curves are drawn through the points.

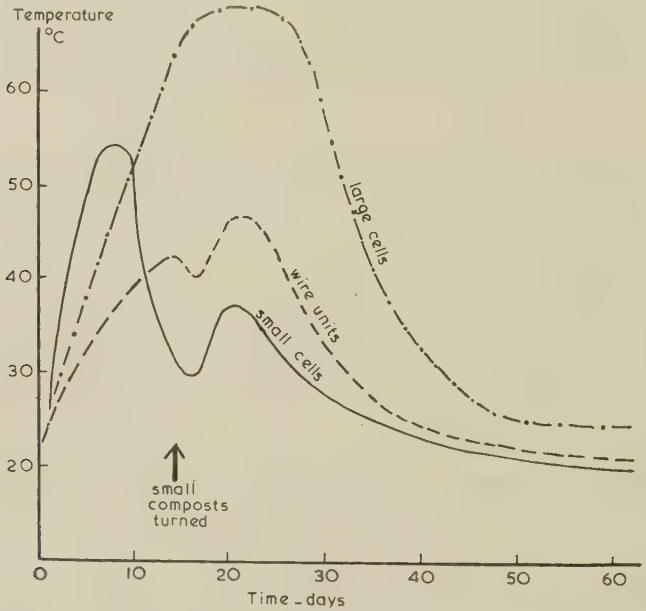

Fig. 1. Time-temperature curves of compost (straw plus 'nitro-chalk', initial C/N ratio 40) in three types of unit. The curves have been smoothed off.

It will be noted that the temperature of the material in the large concrete cells reached a higher value and that this temperature was maintained for a longer period than in the case of the small units.

Where no nitrogen was supplied lower maximum temperatures were attained.

Bacterial counts

In 1954 total bacterial counts of the moist composts in the small concrete cells were made on samples taken when the temperature

of the heaps had reached a maximum. The counts were done by the dilution plate method using Thornton's medium. Results are shown in Table IV.

TABLE IV

Bacterial counts of composts at peak temperature in small concrete units.

Material	Bacterial numbers (millions per g, dry basis)
Straw alone	3,045 ± 279
Straw + nitro-chalk (initial C/N 40)	10,725 ± 495
Straw + nitro-chalk (initial C/N 27)	4,164 ± 456
Straw + grass (initial C/N 40)	7,065 ± 375

Highest bacterial numbers were found where the "optimum" amount of nitrogen had been added, *i.e.* sufficient to give an initial carbon nitrogen ratio of 40. Unfortunately it was not possible to continue studies on the microbiological aspects of composting.

pH measurements

These were made during and at the end of composting of a number of materials in the small concrete cells. Measurements were made with a pH meter either on the moist material shaken with an equal volume of water or on the dried ground material shaken with five times its weight of water. Results are given in Table V.

TABLE V

Results of pH-measurements

Material	Number of days composted		
	7 days	139 days	
	(moist material)	(moist material)	(dried and re-wetted material)
Straw alone	7.61	7.42	7.44
Straw + nitro-chalk (initial C/N 40)	7.40	7.71	7.66
Straw + $(NH_4)_2SO_4$ (initial C/N 40) + 3% $CaCO_3$	—	7.52	7.61
Straw + dried blood (initial C/N 40) + 3% $CaCO_3$	—	7.28	7.22

It will be noted that the pH of all the materials was slightly alkaline at both sampling dates and that the final pH was very

similar for all the accelerators used. Also there was little difference in pH measurements made on the moist or re-wetted materials.

Ammonia and nitrate content of composts

The ammonia and nitrate contents of some of the composts were determined by extraction of the dried ground material with 0.5 *N* sodium acetate followed by distillation-nesslerization (9) for the ammonia and the phenoldisulphonic acid method (8) for nitrate. Results are shown in Table VI.

TABLE VI

Ammonia and nitrate content (ppm dry basis) of composts from small concrete cells (5 months rotting).

Material	NH_3–N	NO_3–N
Straw alone	2	2
Straw + nitro-chalk (initial C/N 40)	2	56
Straw + $(NH_4)_2SO_4$ (initial C/N 40) + 3% $CaCO_3$. .	14	42
Straw + grass (initial C/N 40)	18	36

It will be noted that all the finished composts contained very small amount of extractable ammonia and nitrate as compared with their total nitrogen contents (which range from 17,400 to 19,800 ppm).

DISCUSSION

The unsatisfactory results obtained with the wire units in this study are almost certainly due to excessive losses of moisture and nitrogen during the process, resulting in poor conditions for the micro-organisms which are responsible for the rotting process. It must be pointed out, however, that if much larger open heaps are used satisfatory composting may take place in all parts with the exception of the outer layers. If the surface area is small compared with the volume of the heap then the proportion of poorly rotted material will be low and may be removed and incorporated in the following year's compost heap.

The fact that dry-matter losses and final nitrogen content in the small concrete units were very similar to those in the larger cells, indicates that the former may be used for studying the composting

process. The nitrogen losses (7 to 14 per cent of the initial nitrogen content) are rather higher than migh tbe desired but such losses are inevitable because of the high specific surface of the small heaps.

Because of the difficulty of accurately controlling the moisture content of the heaps no studies were made on the effect of varying moisture content on the composting process. The author considers, however, that a thorough preliminary saturation of the straw with water is essential for satisfactory composting in small heaps. In large heaps where it is not possible to wet the straw uniformly the somewhat poorer composting conditions which might arise are offset by the higher temperatures attained as well as by the fact that this temperature is maintained for a longer period than in the small heaps.

The results obtained in the experiments using varying levels of initial nitrogen confirmed the results obtained by Hutchinson and Richards [8], in that an initial carbon/nitrogen ratio of 40 gives satisfactory composting. With a wider initial carbon nitrogen ratio dry-matter losses were lower, whilst with an initial ratio narrower than 40 there was no more rapid decomposition and the extra nitrogen was merely lost, probably by denitrification.

In this study no attempts were made to control aeration. Aeration arises through convection currents induced by the heating of the compost and serves to remove the large volumes of carbon dioxide which are formed during rotting. Aeration also helps to dissipate the heat evolved during rotting, although part of this is also lost through conduction through the walls and floor of the container. Hoyle and Mattingly [7] concluded that aeration of small cells, by means of land drains placed in the floor, tended to increase rate of decompostion, as indicated by the level of soluble nitrogen in the compost. The differences due to the treatments were small, however, and of doubtful significance. In the small concrete cells used in this study sufficient aeration was obtained through convection currents and leakage of air through the spaces between the concrete slabs.

The final levels of inorganic nitrogen in the composts were very low and did not exceed. 0.5 per cent of the total nitrogen. These levels are much lower than those reported by Hoyle and Mattingly [7] but these workers started with higher initial levels of nitrogen, and this might account for the difference in results.

This slightly alkaline reaction of most of the finished composts

indicate that where alkaline or physiologically alkaline materials such as 'nitro-chalk' or urea are used no extra chalk need be added. No tests were carried out ot find the optimum amount of chalk required when physiologically acid materials such as ammonium sulphate and dried blood were used as accelerators, but the quantity added, viz. 3 lb. calcium carbonate per 100 lb. straw on dry basis, appears to be satisfactory.

CONCLUSION

Satisfactory composting of straw in small concrete cells holding 30 lb. dry straw can be achieved during the period late spring to autumn providing:

(a) The straw is thorough saturated with water prior to adding the accelerator;
(b) Sufficient extra nitrogen is added to give an initial carbon/nitrogen ratio of 40. This nitrogen should be in soluble or readily available form, *e.g.* 'nitro-chalk', ammonium sulphate, dried blood, urea or may be in the form of grass.
(c) The heaps should be mixed two weeks after the start of composting and kept fairly moist by addition of water.

Under these conditions from 50 to 60 per cent of the dry matter is lost over a 4- to 5-month composting period and the final material will have a carbon/nitrogen ratio near 20.

SUMMARY

Straw could be composted satisfactorily in 4 to 5 months in small concrete cells holding 30 lb. material (dry basis), providing the straw was thoroughly moistened and sufficient easily available nitrogeneous material added to give an initial carbon/nitrogen ratio of 40. During the composting process about half the dry matter was lost. Results were similar to those obtained using large cells holding 1500 lb. straw. Wire units holding 100 lb. straw were unsatisfactory. Changes in dry matter, carbon/nitrogen ratio, temperature, soluble inorganic nitrogen, and pH of composts prepared with various accelerators are reported.

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Plant and Soil X, no. 2 December 1958

BEHAVIOUR OF IRON CHELATES IN CALCAREOUS SOILS

III. LABORATORY EXPERIMENTS WITH A FURTHER RANGE OF CHELATING AGENTS

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INTRODUCTION

The behaviour of the iron chelates of six different polyamine-polycarboxylic acids in a highly calcareous soil has already been reported [5 7]. This work has been continued with some new materials suggested for the control of lime-induced chlorosis. Two iron chelates that had previously been reported by other workers to be ineffective against chlorosis have also been included in the experiments to find possible reasons for this behaviour.

The new chelating agents tested are known as RA 155, RA 156, RA 157 and RA 159. The chemical structure of RA 155 is N-(2-hydroxy*cyclo*hexyl)-ethylenediaminetriacetic acid, and hence it is similar to HEEDTA. RA 157 is ethylenediamine N, N'-*bis*-(1-hydroxy-3-methylphenylacetic acid) and is thus of very similar composition to EDHPA. The structures of RA 156 and RA 159 have not been disclosed, but it is known that they contain phenolic groups as do RA 157 and EDHPA. Responses to soil applications of these four compounds have been reported by several workers [1 4 9 11].

Experiments were also carried out with the iron chelates of ammoniatriacetic acid (ATA) and of dihydroxyethylglycine (HEG). Stewart and Leonard [10] found that a calcareous soil adsorbed the iron from these compounds very rapidly, but they did not explain this loss.

The four chelates of the RA series were labelled with Fe^{59} in order to assess the extent of isotopic exchange with soil iron.

Preliminary experiments showed that the recovery of soluble iron from calcareous soils treated with Fe-ATA or Fe-HEG was too small to allow accurate determinations of exchange to be made, hence no isotopic iron was used with these chelates.

EXPERIMENTAL

A full account has been given in a previous paper [5], hence only brief descriptions are given below unless the procedure has been altered.

1. *Soil*

This was an Evesham soil containing 34% calcium carbonate and 33% clay; its pH was 7.8.

2. *Preparation of labelled chelate solutions*

This was done by mixing equimolar quantities of the chelating agents and ferric chloride labelled with Fe^{59}. Before use the excess acidity was neutralized with sodium hydroxide.

3. *Treatments*

Experiment 1. 50-g samples of soil were given one of the following treatments as 20 ml of solution: –

Treatment	Chelating agent	Quantity chelated iron (approx.)	Treatment	Chelating agent	Quantity chelated iron (approx.)
(a)	RA 155	0.5 mg	(e)	RA 157	0.1 mg
(b)	,,	5.0	(f)	,,	0.5
(c)	RA 156	0.1	(g)	RA 159	0.1
(d)	,,	0.5	(h)	,,	0.5
Treatment (i) — Water only					

0.25 μc Fe^{59} was included in the iron chelate solution applied as treatments (a)–(h). The exact amounts of chelate added to the soil are given in the tables as the analytical results for 0 days. These amounts of labelled chelate corresponded to treatment levels of approximately 10 and 100 parts of chelated iron per million of soil for the Fe-RA 155, and approximately 2 and 10 ppm for the other three chelates. The lower levels of treatment were chosen for these three phenolic chelates because, by analogy with the results obtained with Fe-EDHPA [7], they were expected to have a greater stability in calcareous soils.

Until required for extraction, the treated soils were stored at a temperature of between 15° and 20°C, in the dark in glass jars with loose cardboard covers. After each of the four time intervals, (2, 4, 8 and 15 days), three replicates of treatments (a), (b) and (i) were extracted as described below. The replicates of treatments (c), (d), (e), (f), (g) and (h) were extracted after the four intervals 4, 8, 15 and 30 days.

Experiment 2. 50 g samples of soil were given one of the following treatments as 20 ml of solution: –

Treatment	Chelating agent	Quantity chelated iron (approx.)	Treatment level
(j)	ATA	1.25 mg	25 ppm
(k)	,,	5.0	100
(l)	HEG	1.25	25
(m)	,,	5.0	100
(n)	Water only		

No radioactive iron was used in this experiment. Three replicates of each treatment were extracted after each of the four time intervals 2, 4, 8 and 15 days.

4. *Extraction procedure*

The treated soil samples were shaken with 100 ml of 0.4% sodium chloride solution for 30 minutes, then a clear extract was obtained by centrifugation.

5. *Methods of analysis*

a. Soluble iron

(i) Isotope method. The values for soluble iron by the isotope method given in Table II are the amounts of iron that were originally associated with the radioactivity found in the extracts.

(ii) Colorimetric method. The concentration of iron was determined by the *o*-phenanthroline method of Fortune and Mellon [3] with the following modifications to overcome the presence of the chelating agents: —

a) Fe-RA 155, ATA and HEG, – as with Fe-EDTA, the solutions were left for 1 hour after adding all reagents.

b) Fe-RA 157, – as with Fe-EDHPA, 2 ml *N* HCl was added before the *o*-phenanthroline.

c) Fe-RA 156 and RA 159, – 2 ml HCl, the hydroxylamine hydrochloride and the *o*-phenanthroline were added, then the solution heated in a water-bath at approximately 65° C for 30 minutes. After cooling and addition of ammonium acetate buffer, the solutions were left for 1 hour before measurements were made.

(iii) Spectrophotometric method. Experiments on the variation in optical density of chelate solutions with pH [8] showed that the spectrophotometric method for chelated iron described previously [6] could be used for the six substances in these experiments. The optimum wavelengths and pH conditions found are set out in Table I, together with the factors used to convert optical densities into concentrations of chelated iron. It was again assumed that, of the other metals present in these soil extracts, calcium would predominate. At the wavelengths used in this method for iron the absorption due to the calcium chelates was found to be negligible. (See below for special case of results with Fe-HEG.)

TABLE I

Conditions for Spectrophotometric Analysis of Iron Chelates			
Chelating agent	Wavelength	pH range	Regression line factor*
RA 155	262 mμ	2–3	0.126
ATA	260	2–3	0.095
HEG	260	3.0	0.055
RA 156	510	4–7	0.065
RA 157	500	5–10	0.095
RA 159	480	5–7	0.076

* defined as: – Optical density (1 cm) of a solution containing 1 μg chelated iron p. ml

b. 'Total chelate'

The concentration of calcium chelate in the extracts was determined spectrophotometrically as its equivalent of iron chelate by the method described previously [6]. In this the increase in chelated-iron concentration on addition of excess ferric ions is proportional to the concentration of calcium chelate in the original solution. The values given for 'total chelate' in Tables II and III are the sum of the iron equivalent of the chelated calcium and of the iron chelate figures in the adjoining colum.

6. *Presentation of results*

Only 93 ml of solution was recovered from the 50-g soil samples to which had been added 20 ml of chelate solution and a further 100 ml of extractant. In order to allow for the solution left in contact with the soil the figures given in Tables II and III are those obtained from the analysis of the centrifugate multiplied by a factor of 1.3.

RESULTS

In Table II, which contains the results of Experiment I, 'total chelate' values are given for RA 155 only, as the corresponding values for RA 156, RA 157 and RA 159 were approximately equal to the values for soluble iron obtained by the spectrophotometric method.

TABLE II

Chelate content of soil extracts. Experiment 1.
Mean of 3 replicates, expressed as μg Fe.
('Total chelate' values in equivalent of iron).

Iron chelate	Time of contact (days)	Treatment levels: 0.5 mg chelated iron (10 ppm Fe) — Soluble iron: Isotope method	Colorimetric method	Spectrophotometric method	'Total chelate'	5.0m gchelated iron (1000 ppm Fe) — Soluble iron: Isotope method	Colorimetric method	Spectrophotometric method	'Total chelate'
RA 155	0	532	532	562	582	4890	4890	5130	5220
	2	381	401	403	430	3840	4020	3940	3940
	4	350	364	364	420	3470	3680	3610	4120
	8	290	315	325	454	2920	3270	3290	4310
	15	214	230	244	416	2510	2690	2790	4300
		0.1 mg chelated iron (2 ppm Fe) — Soluble iron: Isotope method	Colorimetric method	Spectrophotometric method	'Total chelate'	0.5 mg chelated iron (10 ppm Fe) — Soluble iron: Isotope method	Colorimetric method	Spectrophotometric method	'Total chelate'
RA 156	0	117	117	101		445	445	458	
	4	75	66	69		280	277	296	
	8	66	58	64		266	269	280	
	16	66	53	59		250	239	261	
	30	56	46	52		217	213	232	
RA 157	0	129	129	119		524	524	490	
	4	125	130	118		524	538	480	
	8	114	123	113		494	512	474	
	16	116	120	109		487	493	461	
	30	103	108	98		458	459	430	
RA 159	0	101	101	104		538	538	446	
	4	81	90	92		360	415	382	
	8	74	83	81		317	356	345	
	16	70	76	77		306	333	339	
	30	60	62	66		278	298	315	

It will be seen from Table II that the amount of soluble iron falls at successive times of extraction. After 2 days about 80% of the iron in Fe-RA 155 remains soluble and the recovery falls to about 55% after 15 days. During the same period the 'total chelate' in the extracts is approximately constant at 80% of the iron chelate applied to the soil (Figure 1). These recoveries are about the same

at both treatment levels. The recovery of soluble iron with the chelates RA 156, RA 159 and RA 157 is 65%, 80% and 100% respectively after 4 days, and falls to 50%, 60% and 85% after 30 days. With these three compounds no calcium chelates are found in the soil extracts. The level of treatment makes only small differences to the percentage of added iron recovered.

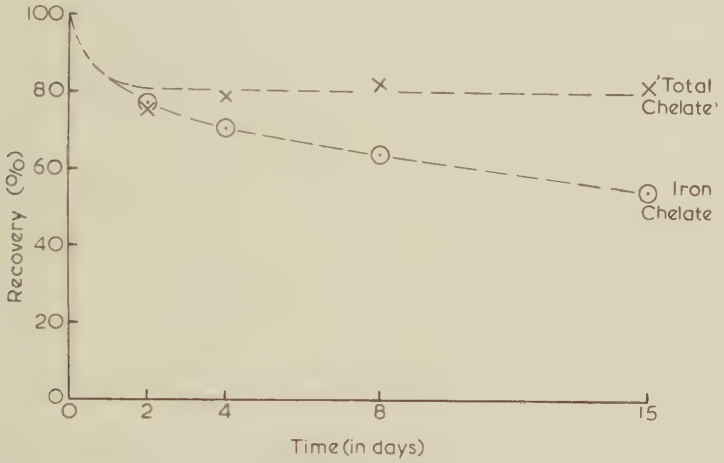

Fig. 1. Effect of time on the recovery of RA 155 from calcareous soil. Approx. 5.0 mg chelated iron added to 50 g soil.

A comparison of the values obtained for soluble iron by the isotope and colorimetric methods on the same extract as given in Table II shows that little or no isotopic exchange has taken place with these four chelates.

Table III gives the results of Experiment 2 and in the case of both Fe-ATA and Fe-HEG the recovery of soluble iron is found to be very low. Only about 1% of the added iron is recovered after 2 days and less than 0.4% after 15 days. The 'total chelate' recovered following Fe-ATA treatment is almost constant at 50% at each sampling time with both levels of application.

Anomalous results were obtained by the spectrophotometric method on the extracts from the soil treated with Fe-HEG. Whereas with other chelating agents there had been reasonable agreement between the figures for soluble iron obtained by the colorimetric and by the spectrophotometric methods, with Fe-HEG the spectro-

TABLE III

Chelate content of soil extracts. Experiment 2. Mean of 3 replicates, expressed as μg Fe. ('Total chelate' values in equivalent of iron)

Iron chelate	Time of contact (days)	Treatment levels: 1.25 mg chelated iron (25 ppm Fe) — Soluble iron: Colorimetric method	1.25 mg chelated iron (25 ppm Fe) — Soluble iron: Spectrophotometric method	1.25 mg chelated iron (25 ppm Fe) — 'Total chelate'	5.0 mg chelated iron (100 ppm Fe) — Soluble iron: Colorimetric method	5.0 mg chelated iron (100 ppm Fe) — Soluble iron: Spectrophotometric method	5.0 mg chelated iron (100 ppm Fe) — 'Total chelate'
Fe-ATA	0	1250	1250	1250	5000	5000	5000
	2	10	9	600	49	90	2300
	4	5	12	630	39	64	2550
	8	2	17	550	34	78	2300
	15	0	12	660	21	55	2740
Fe-HEG	0	1275			5000		
	2	16			166		
	4	7			82		
	8	4			38		
	15	3			22		

photometric results were consistently much higher. A re-investigation was therefore made of some of the 15-day extracts after they had been digested with acid to destroy the chelates. Analyses for iron, copper and manganese were made with the following results: –

Treatment	Soluble constituents μg/100 ml: Iron	Copper	Manganese
(k) Fe-ATA (100 ppm Fe)	18	17	0
(m) Fe-HEG (100 ppm Fe)	19	92	84
(n) Water	4	5	0

It can be seen that the soluble iron figures are in fact very low in the extract from the Fe-HEG treatments, but in this extract a much higher amount of copper and manganese is present than in the other two. As the copper and manganese chelates of HEG absorb light at the wavelength used for Fe-HEG, the spectrophotometric results obtained for iron and 'total' chelate were invalid and have been omitted from Table III.

DISCUSSION

The results of these experiments emphasize the different pattern of behaviour between the iron chelates whose ligands are wholly aliphatic and those containing phenolic groups. Where the chelates are aliphatic, for example with Fe-RA 155 or Fe-ATA, loss of iron from solution can take place by two processes, namely by sorption of the whole iron chelate molecule on to the clay and by replacement of chelated iron by calcium. With phenolic chelates, such as Fe-RA 156, Fe-RA 157 and Fe-RA 159, no replacement of chelated iron by calcium has been detected, although some clay sorption of the whole molecule took place with two of these chelates. These differences can be correlated with the relative stabilities of the iron and calcium chelates formed by the various ligands, as shown by potentiometric titration curves [8]. The ferric iron chelates of RA 155 and ATA have their maximum stability in acid solutions, but form hydroxy-chelates of decreased stability in solutions of the pH of this calcareous soil, (7.8). At this pH, however, their calcium chelates have a moderate stability. In contrast, the phenolic-type iron chelates have a high stability which is undiminished in alkaline solutions below pH 9 to 10; moreover these ligands chelate calcium only weakly.

HEG requires special comment for although it is aliphatic, it too forms only a weak calcium chelate [2]. The almost complete precipitation of the iron from Fe-HEG is probably due more to the low stability of the iron chelate species formed by it in alkaline solutions than to the substitution of the iron by any other metal. However, due to the failure of the spectrophotometric method of analysis, no estimate could be made of the extent of clay sorption nor of the microbiological stability of this chelate in the soil.

Wallace and Shannon [11] left solutions of the four iron chelates of the RA series in contact with both a highly calcareous loam and an acid clay for 5 days. After filtration they recovered almost 100% of the Fe-RA 157 from both soils, and a slightly lower amount of the Fe-RA 159. Only 40% of the Fe-RA 156 was still in solution after contact with the calcareous loam, but about 80% was recovered from the clay. The recovery of Fe-RA 155 was the lowest of the series, about 25% from both soils. This last result is in contrast with the high recovery, about 80%, obtained in our experiments.

On the basis of the recoveries of soluble iron in our experiments, we would estimate that the efficiency of these iron chelates in the control of chlorosis by soil application would be in the order: – RA 157 highly efficient, followed by RA 159, RA 156 and RA 155, all moderately efficient, while ATA and HEG would be completely ineffective. This estimate is in agreement with most reports by other workers of their field and laboratory studies with these compounds. Complete agreement is not expected because of differences in the nature of the soils used, especially the pH, the $CaCO_3$ content and the types of clay minerals present.

Gasser and Müller [4] reported a rapid response in young chlorotic pears to very low amounts of Fe-RA 157 applied to the soil, (1 g iron per tree). They found that similar amounts of Fe-RA 159 were much less effective.

In field trials with mature citrus trees of between 12 and 28 years old, Kuykendall *et al.*[9] found that Fe-RA 159 was largely ineffective in controlling chlorosis even when 48 g chelated iron per tree were washed into the soil. Fe-RA 157 applications controlled the chlorosis, but this chelate was slower to act and was less efficient than the closely related compound Fe-EDHPA.

Bould [1] found that much lower amounts of Fe-RA 157, 2 g iron per small tree, controlled chlorosis in pears, but also noted that this chelate was slightly less effective than the same amount of Fe-EDHPA.

From pot tests with soyabeans growing in a highly calcareous soil, Wallace and North [11] found that at 2.5 ppm Fe-RA 157 or Fe-RA 159 in soil gave the best responses as judged by increased dry weights and reduction in chlorosis. In the same test Fe-RA 156 gave a smaller response, while the Fe-RA 155 treatment resulted in no increased growth over untreated controls. Higher rates of application, 100 ppm, were very toxic when applied as Fe-RA 155 and also, to a lesser extent, when given as Fe-RA 159.

It is of interest that as in previous experiments in this series [5 7], the percentage recovery of 'total chelate' has been found to be roughly the same irrespective of the rate of application.

The results from Experiment 1 show that no appreciable isotopic exchange took place between the four labelled chelates of the RA series and iron in this soil. The differences between the results by the isotope and the colorimetric methods are probably due to slight

errors in the analysis of the original solutions. Isotopic exchange may take place with these compounds under different soil conditions.

SUMMARY

A highly calcareous clay soil was treated with six iron chelates, those formed by RA 155, RA 156, RA 157, RA 159, ATA or HEG, and then extracted with an aqueous solution after intervals of up to 30 days.

About 80% of 'total' RA 155 was recovered at each sampling time, but after 15 days approximately one-third of the iron in it had been replaced by calcium. Some of the Fe-RA 156 and Fe-RA 159 was sorbed by the clay, but no calcium was found to have replaced iron chelated with RA 156, RA 157 or RA 159.

The four chelates of the RA series were labelled with Fe^{59}. The amount of isotopic exchange taking place between the Fe^{59} and soil iron was found to be negligible.

A very low percentage of Fe-ATA and Fe-HEG was recovered from the soil. Approximately 50% of the Fe-ATA was sorbed by the clay, and in the remainder the chelated iron was replaced by calcium. The cause of the loss of Fe-HEG could not be determined.

ACKNOWLEDGEMENTS

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LETTER TO THE EDITORS

The effect of sodium on some histological characteristics of flax stems

Introduction

In a previous paper, Lehr and Wybenga demonstrated the influence of sodium on the yield and quality of flax fibres [1].

Their results, obtained in a series of pot experiments with flax, were summarized as follows: "Sodium, applied either in the nitrate form or as dusarit in an artificial soil complex, stimulated the vegetative development, even in the presence of an ample K-supply. It also had a favourable effect on the quality of the fibre, which was greatest when 6 per cent of the exchangeable bases were in form of sodium but gradually decreased at higher percentages".

When discussing these results, the hypothesis was put forward that under the influence of different contents of sodium certain histological modifications take place in flax plants, especially in the fibrous tissue. A new investigation, carried out by us in 1955 to test this hypothesis, is reported in this article.

Experimental section

The experiment was carried out on mixtures of dusarit-quartz sand and the experimental technique was exactly the same as in Experiment C of the publication already cited [1].

Pots were in duplicate with 30 plants per pot (variety "Concurrent"), and were sown on the 28th April 1955. Five sodium levels were included, viz. 0, 3, 6, 12 and 18% Na-dusarit; all mixtures contained 6% K-dusarit and 6% Mg-dusarit and were made up to 100% with Ca-dusarit.

The plants were harvested on the 11th of July. Portions of the stems were taken at heights of 10 cm and 28 cm from each plant of each treatment. The cuttings, fixed in Karpeschenko, were then enclosed and dissected.

Results

On examination the various histological preparations showed differences of some significance in the structure of the fibrous bundles. These differences were related to the quantity of sodium administered, and were, in particular, to be found at the lower level, where the tissues were older and therefore more differentiated. (See Plates.)

In brief, the main points of the investigation can be summarized as follows:

1. The differences in histological analysis due to sodium are technologically favourable up to a maximum of 6% sodium; above this level, the histological structure becomes gradually less favourable technologically with increasing sodium percentages until, at 18% sodium, it is nearly the same as that of the control,
2. The favourable effect of the differences found, lies in the uniformity of development and differentiation of the fibres, and in the greater thickness of the cell wall of the single fibres,
3. The harmful effect, when an excess of sodium is present, lies in the discontinuity of the fibrous ring, in the thinning and deformity of the cell walls of the single fibres, many of which are underdeveloped, and in the wider lumen which they show in consequence of the thinner cell walls.

The present investigation was limited to the fibrous tissues of the flax stem; however, changes in the structure of other tissues may also occur.

Discussion

It can be concluded from a comparison of the present results with those obtained in the experiments carried out by Lehr and Wybenga in 1952 that the histological analysis of flax plants grown in the presence of sodium reflects their cultural treatment. The favourable influence of sodium on the development and quality of the flax fibres is evident.

Summary

An investigation was carried out on the effect of sodium on the histological structure of flax stems. The plants were grown on an artificial soil with sodium levels varying from 0 to 18% of the adsorbed cations. Potassium and magnesium were kept at a constant level. Observation of the histological preparations confirms former findings that the formation and the quality of the fibre respond favourably to sodium. The effect is optimal in a treatment with 6% of sodium; at higher levels it gradually decreases and is negligible at a level of 18% Na.

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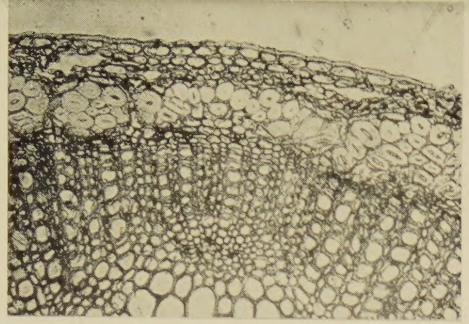
a.



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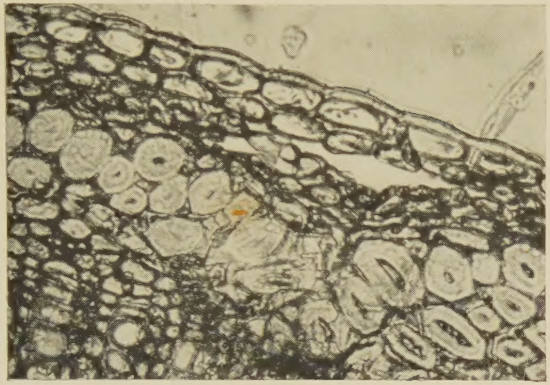
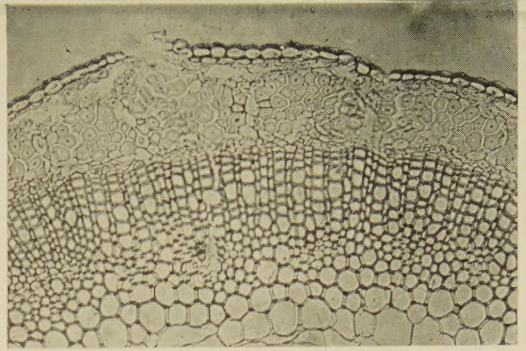
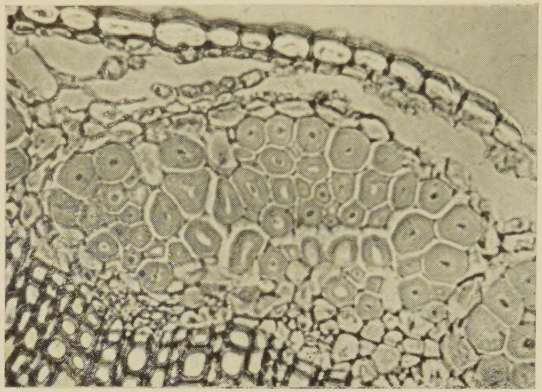


Plates I[a] and I[b]. Cross section of the stem of a flax plant, grown *without sodium.* Note the irregular growth of fibrous tissues and reduced number of cells which are often deformed (Magnification I[a] 150 ×, I[b] 375 ×)

a.



b.



Plates II[a] and II[b]. Cross section of the stem of a flax plant, *with 6% sodium* in the adsorptive complex. Note the better development of the fibrous ring with increased number of cells, usually of a regular shape. (Magnification II[a] 150 ×, II[b] 375 ×)